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LIVER INJURY AND LIVER CIRRHOSIS

An Experimental and Clinical Study

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by

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بسم الله الرحمن الرحيم
وما أوتيتم من العلم الا قليلا

Of Knowledge It Is Only A Little That is Communicated

To You (O men!)

يؤتي الحكمة من
يشاء ومن يؤت
الحكمة فقد أوتي خيراً
كثيراً وما يذكر الا
اولوا الالباب

He Granteth Wisdom
To Whom He Pleaseth;
And he to whom wisdom
Is granted receiveth
Indeed a benefit overflowing;
But none will grasp the Message
But men of understanding

Holly Koran

صدق الله العظيم

To my God Father, Hamzah Al-Salman,

Talib Mansoor, Hassan and Abdul-Rehman Dashti,

Sadeq Al-salman and to Ali, Mohammed and my wife

The thesis is based on the following papers; which are referred to in the text by their Roman numerals.

I. Early biochemical and histological changes in rats exposed to a single injection of thioacetamide. H. Dashti, B. Jeppsson, I. Hägerstrand, B. Hultberg, U. Srinivas, M. Abdulla, S. Bengmark.
Submitted for publication.

II. Changes in the plasma levels of copper, zinc, calcium, magnesium and selenium in thioacetamide induced liver cirrhosis. H. Dashti, B. Jeppsson, B. Hultberg, I. Hägerstrand, M. Abdulla, U. Srinivas, S. Bengmark.
Acta Pharmacologica et Toxicologica. Accepted for publication.

III. Thioacetamide and carbon tetrachloride-induced liver cirrhosis. H. Dashti, B. Jeppsson, I. Hägerstrand, B. Hultberg, U. Srinivas, M. Abdulla, S. Bengmark.
Submitted for publication.

IV. A simple method for producing liver cirrhosis in the rat. H. Dashti, B. Jeppsson, I. Hägerstrand, B. Hultberg, U. Srinivas, M. Abdulla, S. Bengmark.
Submitted for publication.

V. Serial hepatic resections in cirrhotic rats. H. Dashti, B. Jeppsson, I. Hägerstrand, U. Srinivas, M. Abdulla, S. Bengmark.
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VI. Effect of zinc administration on CCl_4 -induced liver cirrhosis. H. Dashti, B. Jeppsson, I. Hägerstrand, B. Hultberg, U. Srinivas, M. Abdulla, S. Bengmark.
Submitted for publication.

VII. Zinc, liver cirrhosis and hepatic encephalopathy. B. Jeppsson, H. Dashti, B. Hultberg, P. Herlin, U. Åslund, B. Joelsson and S. Bengmark.
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VIII. The role of free radicals in the pathogenesis of alcohol, carbon tetrachloride and thioacetamide induced cell injury. H. Dashti, G.G. Jonsson, B. Jeppsson, R. W. Pero, S. Bengmark.
Submitted for publication.

IX. The role of superoxide dismutase in the prevention of CCl_4 -induced liver injury. H. Dashti, B. Jeppsson, G.G. Jonsson, I. Hägerstrand, R.W. Pero, T. Strohmeyer, B. Hultberg, S. Bengmark.
Submitted for publication.

ABBREVIATIONS

ASAT	Aspartate aminotransferase (L-Aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1)
ALAT	Alanine aminotransferase (L-Alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2)
ALP	Alkaline phosphatase (Orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1
GT	γ-Glutamyltransferase (Glutamine:D-glutamyl-peptide glutamyltransferase, EC 2.3.2.1)
β-Gluc	β-D-Glucuronidase (β-D-Glucuronide glucuronohydrolase, EC 3.2.1.31
β-NAG	β-N-Acetylglucosaminidase (2-Acetamido-2-deoxy-D-glucoside acetamidodeoxygluco-hydrolase, EC 3.2.1.50)
NAD	Nicotinamide adenine dinucleotide
GSH	Glutathione peroxidase
SOD	Superoxide dismutase
O_2^-	Superoxide
H_2O_2	Hydrogen peroxide
OH^-	Hydroxyl radical
CCl_4	Carbon tetrachloride
CCl_3	Trichlormethyl radical
DMNA	Dimethylnitrosamine
ADPRT	Adenosine diphosphate ribosyl transferase
DNA	Deoxyribonucleic acid
^3H -dThd	Methyl- ^3H-thymidine (tritiated thymidine)
^3H -NAD^+	Adenine- ^3H-nicotinamide adenine dinucleotide
TCA	Trichloroacetic acid
S.D.	Standard deviation

SEM	Standard error of the mean
I.P.	Intraperitoneal
I.G.	Intragastrical
Thioac	Thioacetamide
L.P.	Lipid peroxidation

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To a man who knows nothing
Mountains are mountains
Water is water and
Trees are trees.
When he has studied and
knows a little.
Mountains are no longer mountains
Water is no longer water and
Trees are no longer trees.
When he has thoroughly understood,
Mountains are again mountains
Water is water and
Trees are trees.

Anonymous

INTRODUCTION

Liver disease in many countries is a leading cause of death. In the western world alcoholic liver disease is increasing due to increasing alcohol consumption (Saunders et al, 1981). According to Galambos, alcoholic cirrhosis is one of the leading causes of death in affluent countries (Galambos, 1979). Rather recent epidemiological studies have shown clearly that alcoholic liver injury and cirrhosis is related to the dose and duration of alcohol intake (Pequinot et al, 1974). There is, however, no correlation between alcohol consumption and the relative frequency in the development of liver cirrhosis. It has been estimated that while 30% of chronic alcoholics develop hepatitis at some stage of their lives, only 10% of them develop cirrhosis (Lelbach, 1976). Besides alcohol consumption, nutritional, genetic and immunological factors play important roles in the development of liver cirrhosis. Hepatitis B virus infection

is an assumed risk factor predisposing alcohol abusers to develop liver cirrhosis. It has also been shown that progressive liver damage can be caused by this virus (Sherlock et al, 1970). An indication of such correlation is the frequent presence of HBeAg in sera of patients suffering form chronic active hepatitis and cirrhosis (Kelker et al, 1973a., 1973b; Tandon et al, 1979).

There are probably also other yet unidentified environmental factors contributing to the progression of chronic liver disease in alcoholics. Alcohol consumption, thus, has become one of the most challenging health hazards of the present time. The rehabilitation of alcoholics with liver cirrhosis places enormous demands on the economical resources of the society.

So far no effective measures are available for the treatment of liver cirrhosis. The different surgical and medical therapeutic manouvers used at present are only palliative in nature and the results are generally poor. It is important, therefore, especially from a public health point of view, to recognise the chronic alcoholic who is at risk of developing cirrhosis, the progression of which may therefore, be possible to stop.

Alcoholic liver cirrhosis alters the metabolism of many

nutritional factors (Sherlock, 1984), e.g. that of trace elements such as zinc, copper, magnesium, calcium and selenium (Vallee, 1956; 1957; Prasad, 1983; Dashti et al, 1984: Aaseth, 1982). The significance of trace element changes in liver disease is not clear at present. No systematic studies have so far been carried out to elucidate the significance of changes of trace elements in the liver and plasma during the development of liver cirrhosis.

A. LIVER INJURY AND CIRRHOSIS

Cirrhosis is a chronic disease of the liver in which a diffuse destruction of hepatic parenchymal cells occurs, and in which a diffuse increase in connective tissue results in disorganization of the lobular and vascular architecture (Conn, 1982). The pathogenetic mechanisms underlying the development of liver cirrhosis are not fully understood. The present work discusses some important factors, especially relevant in the development of alcoholic liver cirrhosis.

1. Direct effect of alcohol.

Alcohol and its metabolites seem to have direct effects on the hepatocyte function (Rubin and Cederhaum, 1977), but indirect (ischemia) and immunological mechanisms may play

even more important roles in the development of alcohol induced hepatocyte damage and production of fibrosis (Neuberger et al, 1984). An increased incidence of autoantibodies in patients with alcoholic cirrhosis has been demonstrated (Baily et al, 1976). In addition, acetaldehyde, a toxic metabolite of alcohol degradation depresses liver glutathione levels and inhibits protein synthesis (Lieber, 1980). It has, however, been observed that patients consuming Antabuse (disulfiram) to reduce alcohol intake have high levels of acetaldehyde in their blood with no evidence of side effects indicating that acetaldehyde is probably not a primary factor in the etiology of cirrhosis (Lewis and Paton, 1982). It is also likely that alcohol may act synergistically with other factors, e.g. endotoxins, free radicals or trace elements (see below).

2. Role of Endotoxins.

Endotoxin is normally present in the portal venous blood (Jacob et al, 1977). It has been shown that systemic endotoxaemia can occur in patients with liver diseases (Prytz et al, 1976; Jacob et al, 1977; Wilkinson et al, 1980).It has also been suggested that enteric microbial agents or their toxins are responsible for the progess of liver cirrhosis in rats with nutritional deficiencies (Salmon and Newberne, 1962; Broitman et al. 1964). This is

supported by experiments by Nolan and Leibowitz (1978) who showed that pretreatment of animals with the antibiotic polymyxin B protects these animals against the lethal and sublethal effets of CCl_4 possibly by reducing the endotoxin content. However, the exact mechanism whereby endotoxin may influence hepatic function, or induce cell necrosis and collagen formation is unclear. It has been shown that endotoxin activates blood coagulation mechanism leading to local or diffuse intravascular thrombosis (Corrigan et al, 1971), and thus may lead to hepatic ischaemia. The role of this in the development of cirrhosis is unclear at the moment. Endotoxin are therefore only probably partially involved in the pathogenesis of liver cirrhosis.

3. Role of free radicals.

Free radicals are now considered to be involved in cellular damage in many organ systems (Bulkley, 1983). There is evidence that oxygen free radicals reaction also may play a role in cellular damage in alcoholic liver diseases (Lewis and Paton, 1982). A decrease in the glutathione peroxidase levels (GSH) and increase in lipid peroxidation (L.P.) have been observed in man and animals after the administration of alcohol (Videla and Valenzuela, 1982). Peroxides in the serum and liver tissue have also been observed elevated in alcoholic patients when compared to that of control

patients.

Oxygen free radicals such as superoxide(O_2^-) and hydroxyl radicals (HO^-) stimulate lipid peroxidation which leads to cell damage 6 and cell necrosis (Plaa and Witschi, 1976).

Furthermore, free radicals damage intracellular structures such as lysosomes and microsomes (Chein and McCay, 1972; Fong et al, 1973; Wills, 1969; Pryor, 1973; Bors et al, 1974; Salden et al, 1980) and interact with polyunsaturated fatty acids to initiate a complex series of reactions (Slater, 1984).

Lipid peroxidation has been shown to damage isolated hepatocytes (Arundi et al, 1979; Kappus et al, 1979). Shaw et al (1981) showed that lipid peroxidation level was enhanced after ethanol administration to animals, and it was suggested that lipid peroxidation may play a role in the pathogenesis of alcoholic liver disease through the action of free radicals (Shaw et al, 1981). It was suggested by Comporti et al (1973) that lipid peroxidation is enhanced after ethanol intoxication. Furthermore, the glutathione content and the lipid soluble antioxidant level of the liver are reduced after acute ethanol intoxication (Comporti et al, 1973; Di Luzio and Hartman, 1967). Lieber and Decarli (1970) found that the activity of both H O and NADPH

oxidase of the liver microsomes were enhanced following the administration of alcohol to rats. They suggested that H_2O_2 and NADPH oxidase causes lipid peroxidation and glutathione peroxidase (GSH) prevents lipid peroxidation of mitochondria and microsomes (Comporti et al, 1973).

Lewis and Paton (1982) hypothesised that repeated liver damage due to consumption of alcohol could be caused by generation and disposition of superoxides, either by increased conversion of xanthine dehydrogenase to oxidase or through an abnormality of hepatic superoxide dismutase.

The suggestion of a role of free radicals in the pathogenesis of liver cirrhosis is of importance also from a therapeutic point of view. The administration of scavangers might modify the cellular damage. It would be of great interest to study if similar reactions can be elicited by other hepatotoxins and then study the effect of scavangers in experimental models of liver cirrhosis.

In the future it may be possible to identify people with deficiencies in scavenging functions (e.g. ADPRT, see below) and thereby sensitive to free radical induced liver injuries.

4. Role of trace elements.

During recent years an increased knowledge about trace element changes in various disease states has been reached. Trace elements participate as metalloenzymes in many important metabolic functions. Since the liver is the main storage for many trace elements, e.g. zinc and copper, profound alterations are seen in various liver diseases. The changes of zinc and copper may not only be secondary to liver disease, but may also play a direct pathogenetic role. I will focus on zinc and copper and discuss their functions in the body with special reference to the pathogenesis of liver injury and liver cirrhosis.

4.1 Zinc.

Zinc plays an important role in various functions in the body such as immunity, wound healing, growth, reproduction, protein and carbohydrate metabolism (Prasad, 1983; Biesel, 1977). In addition, abnormalities of taste, loss of hair, dermatitis, neuropsychiatric symptoms, failure to grow and hypogonadism have been associated with zinc deficiency (Prasad, 1963a, 1963b, 1963c).

Zinc is important for the normal function of the immune system through its involvement in enzymes, humoral mediators

and its mitogenic effect (Bach, 1981). Zinc deficiency seems to predispose to various infections (Chandra, 1983; Chandra et al, 1985). It has been shown in in-vitro studies that zinc may have mitogenic activity; and lymphocyte cultures can be stimulated by zinc to increase DNA and RNA synthesis (Berger and Skinner, 1974).

Zinc is mainly absorbed from the small intestine, and the amount of zinc absorbed is dependent on the concentration of zinc in the intestinal mucosa which in turn is dependent on the level of zinc in the plasma (Evans et al, 1973). There seems to be a negative feedback i.e. absorption of zinc decreases whenever the plasma level is high and vice versa.

After absorption, zinc is transported to the liver via the portal circulation (Evans, 1976). In plasma, albumin is the major transport protein for zinc although 30-40% of serum zinc is tightly bound to α_2-macroglobulin (Parisi and Vallee, 1970). It has also been found that 2-8% of serum zinc is ultrafiltrable, and that a large amount of this is probably bound to the amino acids histidine, glutamine, threonine, cysteine and lysine. These amino acids compete with serum protein for zinc binding (Prasad and Oberleas, 1970). High zinc concentrations are found in the liver, bone, muscles, skin, prostate, kidney and hair.

Zinc plays an essential role in the synthesis of nucleic acids and proteins and in cell division (Vallee, 1976). It forms an integral part of more than 70 enzymes such as alkaline phosphatase, glutamic dehydrogenase, carbonic anhydrase, carboxypeptidase and RNA polymerase (Kirchgessner et al, 1976). It also stabilizes biological cell membranes by decreasing lipid peroxidation and inhibiting lysyl oxidase in vitro (Chvapil, 1973).

Zinc has a protective effect against membrane peroxidation induced by heavy metals, carbon tetrachloride and high oxygen tension, (Bettger and O'dell, 1981; Chvapil et al, 1972; 1974; Chvapil, 1973; Bettger et al, 1978).

Chvapil (1973) suggested that zinc interferes with the oxidation of NADPH, which inhibits the sequence of reaction in lipid peroxidation. He concluded that dietary zinc controls lipid peroxidation in the liver. Furthermore, superoxide dismutase, which is a zinc metalloenzyme, plays an important part in protecting tissues against superoxide anion (Fee and Teitelbaum, 1972; Chvapil, 1973). Administration of zinc inhibits the formation of malondialdehyde, decrease the accumulation of collagen in the liver and stabilizes lysosomal membranes in animals with carbon tetrachloride poisoning (Chvapil et al, 1972; Anttinen et al, 1984). Zinc also stabilizes the platelet

membrane (Chvapil et al, 1977; 1975). It has been found that administration of zinc salts protects rat liver from the acute hepatotoxic effects of carbon tetrachloride (Voigt and Saldeen, 1965; Saldeen and Brunk, 1967; Saldeen, 1969; Srinivasan and Balwani, 1969). It is not known if zinc has any effect on cirrhosis formation after CCl_4 administration, and it is unknown if zinc modulates the effect of other hepatotoxins as well. However, the fact that it stabilizes membranes, protects against superoxides and reduces fibroplasia makes this very likely and of interest to study.

During liver regeneration liver zinc content rises rapidly and reaches its maximum level within 14 h and declines to its normal value at 48 h (Chain and Griffiths, 1984). The regenerative capacity of the cirrhotic liver and its regulation is not fully known. As trace elements are known to be important in collagen synthesis, DNA synthesis, protein metabolism and the liver is important for these processes, we found the study of trace element changes in regenerating cirrhotic livers interesting.

4.1.1. Superoxide dismutase (SOD).

Superoxide dismutase (SOD) is an enzyme thought to be present in all oxygen metabolising cells and is necessary for their survival (Fridovich, 1975). Zinc and copper are

necessary for the action of SOD and the Zn-Cu-SOD is found in animals, fungi and plants. Superoxide radicals and SOD play a role in acute and chronic inflammation, phagocytosis, radiation damage, arthritis, aging and cancer (Huber and Saifer, 1977; Curnette et al, 1974; Babior, 1978; Oberley et al, 1976; Floe et al, 1980; Tolmasoff et al, 1980).

Superoxide dismutase is a scavanger of superoxide anion radical which is produced during inflammatory processes. SOD scavanges (O_2^-) radicals through the following reaction: $O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$ (McCord and Fridovich, 1969). The one-, two-, or three-electron reduction of molecular oxygen gives rise to superoxide (O_2^-), hydrogen peroxide (H_2O_2), or hydroxyl radical (OH^-), respectively (McCord, 1983) (fig.1). Superoxide is a good reducing agent and a mild oxidising agent, and can initiate free radical chain reactions.

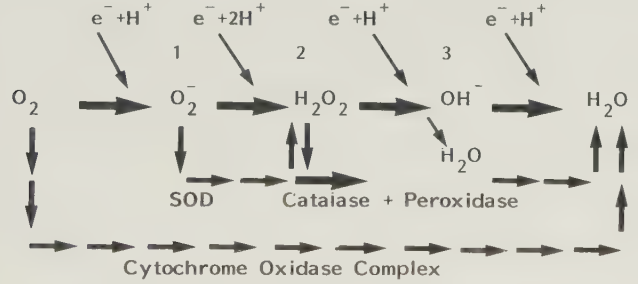


Fig.1. Reduction of O_2 to H_2O requires addition of electrons. Cytochrome oxidase system, which consists of SOD, catalase and peroxidase, is needed for the reduction of O_2. 1. superoxide radical; 2. hydrogen peroxide; 3. hydroxyl radical.

The production of superoxides is stimulated by foreign compounds leading to intracellular killing of microorganisms, lipid peroxidation, tissue damage, release of lysosomal enzymes, and prostaglandin biosynthesis (Babior et al, 1975; Thomas et al, 1978; Pfeifer and McCay, 1971; Fong et al, 1973; Pangamala et al, 1974). It has been observed that Zn-Cu-SOD and Mn-SOD are markedly increased in patients with renal and liver disease (Nishimura et al, 1982a; 1982b).

The mechanisms of the anti-inflammatory activity of superoxide dismutase appears to be the prevention of the activation of superoxide-dependent chemotactic factors (McCord, 1983).

The increased capillary permeability induced by ischaemia is significantly reduced by SOD (Granger et al, 1981), furthermore, SOD prevents the denudation of villi, decreases the necrosis of villi and crypt epithelium produced by regional hypotension (Parks et al, 1982). Manson et al (1983) found that pretreatment of the animals with SOD significantly enhanced island skin flap survival from 0% to 53%.

4.2. Copper.

In plasma approximately 90% of copper is found in ceruloplasmin, 5-10% is loosely bound to albumin and amino acids such as histidine, threonine and glutamine (Bremner, 1980). Copper is absorbed from the stomach and upper gastrointestinal tract. The absorption is faciliated when copper is bound to amino acids and proteins such as superoxide dismutase and metallothionein (Burch et al, 1975).

It is possible that cells take up ceruloplasmin and degrade it so that copper can be obtained (Halliwell and Gutteridge, 1985). The major portion of copper in erythrocytes occurs as the enzyme superoxide dismutase, which is also present in liver and brain (Burch et al, 1975). Copper is often bound to cytosolic protein and metallothionein (Bremner, 1979). In the liver it is incorporated into the glycoprotein ceruloplasmin which is non-exchangeable.

The amino acid complex may be important for the entry of copper into cells and cellular compartment (Burch et al, 1975). Copper is mainly excreted in bile, where it is primarily bound to protein macromolecules (Adelstein and Vallee, 1961; Burch et al, 1975).

Copper participates as a component of ceruloplasmin, ferroxidase II, lysyl oxidase, monoamino oxidase, Zn-Cu-superoxide dismutase, tyrosinase, dopamine beta-hydroxylase and cytochrome-c-oxidase (Hsu, 1980). It thereby participates in erythropoiesis and leukopoiesis, bone mineralization, elastin and collagen cross-linking, oxidative phosphorylation, catecholamine metabolism, melanin formation and antioxidant protection of cells (Mason, 1979; Solmons, 1979; Hsu, 1980).

Copper deficient newborn lambs develop anaemia, leukopenia, hypopigmentation, ataxia with defective myelination, abnormal bone formation with spontaneous bone fractures and cardiac aneurysms (Underwood, 1977; O'Dell et al, 1961; Shields et al, 1962). In humans hypochromic anaemia and neutropenia have been observed (Mason, 1979).

Hepatic copper content increases in various inflammatory conditions, liver cirrhosis and in Wilson's disease (Owen jr, 1980). Increased liver copper content has also been found in patients with primary biliary cirrhosis, long standing biliar obstruction, viral hepatitis and chronic active liver disease (Ritland et al, 1977; Henkin and Smith, 1972). Copper availability is essential for the synthesis of mature collagen and elastin and impaired collagen synthesis has been described in copper deficient animals (Harris et

al, 1980; Giamaolo et al, 1982).

4.2.1. Copper and free radicals.

Copper protects against free radical induced cell injury through its participation in Zn-Cu-SOD. It is however, also possible that bound copper ions can generate OH^- radicals which react immediately with the binding molecule. This has been suggested to occur in Wilson's disease (Halliwell and Gutteridge, 1985). This is a congenital disease inherited as an autosomal recessive trait and is characterized by mental deterioration, ataxia and hepatic necrosis, which results in release of large amounts of copper from degenerating hepatocytes into the plasma leading to haemolysis and jaundice (Roche-Sicot et al, 1973). The pathological changes primarily occur in the liver where fatty degeneration of hepatocytes, necrosis and postnecrotic cirrhosis develop (Sternlieb, 1972; Sternlieb and Scheinberg, 1984). The toxicity of copper affects the central nervous system, kidney and cornea (Sternlieb, 1966; Sussman and Scheinberg, 1969). The level of ceruloplasmin is usually low, serum copper content and copper excretion in urine are high (Ritland et al, 1977). Copper catalyses the oxidation of non-protein SH-groups, and this may give rise to the tissue damage (Ritland et al, 1977), Consistent with this mixtures of Cu^+ and H_2O_2 have been observed to damage many proteins

(Halliwell and Gutteridge, 1985).

Administration of zinc to patients with Wilson's disease led to copper deficiency through the inhibition of copper absorption from the gastrointestinal tract (Brewer et al, 1983; Prasad and Oberless, 1970). According to Brewer et al (1983) zinc administration to patients with Wilson's disease produced a negative to neutral copper balance in all 5 patients. They concluded that zinc therapy can be offered as an alternative to penicillamine therapy.

Copper is also considered to play a pathogenetic role in primary biliary cirrhosis. This is a condition with an obstructive type of jaundice in middle-aged and older females. The exact aetiology of this disease is unknown, although immune complexes were postulated (Sherlock, 1982b).The disease is characterized by destructive cholangitis and periportal hepatitis which leads to cirrhosis and portal hypertension (Sherlock, 1982b). It has been shown that copper levels increase with the duration of the disease and this may reflect the severity of cholestasis (Benson, 1979). Penicillamine, which has been used in the treatment of primary biliary liver cirrhosis, has side effects such as nausea, vomiting, proteinuria, lymphoadenopathy, thrombocytopenia, rashes and transient taste loss (Leeson and Fourman, 1967; Conn, 1982). Death

results from liver failure or from portal hypertension. Since copper deposition in the liver leads to cirrhosis, zinc therapy has been tried. In the study performed by Olsson (1982) no effect of zinc therapy in the treatment of primary biliary cirrhosis could be observed however.

Another condition considered to be caused by excess deposition of copper in hepatocytes is Indian childhood cirrhosis (infantile hepatic cirrhosis) (Portmann et al, 1978; Tanner et al, 1979; Popper et al, 1979).

4.3. Other trace elements.

4.3.1. Selenium promotes growth, prevents liver necrosis and degenerative white muscle disease in animals (Schwartz and Foltz, 1956; Nichoalds, 1984). Schwartz (1961) noted growth response in patients suffering from Kwashiorkor disease after selenium supplementation.

Selenium is known to be a component of the membrane protecting enzyme glutathione peroxidase which has the ability to convert lipoperoxidase to nontoxic substances (Diplock, 1976; Aaseth et al, 1980). It is also an important enzyme in destroying hydroperoxides. Therefore, it protects the cell membrane from oxidative damage. Experimental studies have shown that dietary deficiency of selenium leads

to liver necrosis in rats and pigs (Chernick et al, 1955; Schwartz and Foltz, 1956). Aaseth et al (1982) found that the serum selenium level was 73 and 80 percent below normal in patients suffering from chronic active hepatitis and alcoholic cirrhosis, respectively. They concluded that selenium deficiency may enhance the development of liver cirrhosis (Aaseth et al, 1980).

Low serum selenium levels in humans with alcoholic liver cirrhosis could be secondary to the reduced hepatic synthesis of plasma proteins. This decreases the binding capacity of selenium to α_2-globulin. Decreased dietary intake in combination with increased urinary excretion of selenium may also contribute to the low serum selenium levels in alcoholic patients (Aaseth et al, 1980).

4.3.2. Phosphorous is included in the structure of many cellular elements and enzymes. It is a major intracellular anion. Most of intracellular phosphorous is in organic form and a small proportion is inorganic.

Phosphorous plays an important role in the defence against infectious organisms (Knochel, 1977). Hypophosphataemia was found in patients with bacteremia due to gram-negative organisms (Riedler and Scheitlin, 1969).

In almost all chronic alcoholics, the phosphorous content in muscles was found to be low, a factor probably adding to the morbidity in long term alcoholics (Knochel, 1975; 1980). Ingestion of large quantities of ethanol results in hypophosphataemia (Matter et al, 1964).

It has been suggested that hypomagnesaemia in alcoholic patients is responsible for the increased urinary excretion of phosphorous resulting in low contents of magnesium and phosphorous resulting in low contents of magnesium and phosphorous in skeletal muscles (Whang and Welt, 1963; Knochel, 1980). According to Knochel (1977) there is a relationship between hypophosphataemia, hypomagnesaemia and hypocalcaemia. It has been suggested that hypophosphataemia may be responsible for alcoholic myopathy (Knochel, 1975; Ryback et al, 1980). In addition to alcohol, there are many contributing factors in the development of hypophosphataemia, such as malabsorption, starvation, vomiting and intravenous administration of glucose and saline (Guillou et al, 1976; England et al, 1979).

Low serum phosphorus levels reduce the content of 2.3 di-phosphoglycerate and adenosine triphosphate in red blood cells and hypophosphataemia may produce a severe haemolytic anaemia which contributes to tissue hypoxia (Travis et al, 1971; Jacob and Amsden, 1971; Klock et al, 1974; England et

al, 1979).

5. Immunological factors.

It has been suggested that cellular immunoreactivity may play a role in the pathogenesis of Schistosomal liver disease, chronic active hepatitis and primary biliary cirrhosis (Warren, 1972; Wolfson et al, 1972; Smith et al, 1972; Giustino et al, 1972). Furthermore, progressive destruction of hepatocytes and development of cirrhosis in patients with alcoholic hepatitis could be related in part to immunological hyperactivity (Leey et al, 1975). Elevations of IgG and IgA correlated with the presence of cirrhosis in one study and it was suggested that this hyperglobulinaemia resulted from proliferation of Kupffer cells and thus facilitating B cell function (Wybran and Govaerts, 1975). Others have suggested that the increase in immunoglobulin concentration in alcoholic cirrhosis results from shunting in collaterals and thereby allowing antigens derived from the gut to bypass the liver and stimulate production of antibodies in the spleen (Triger et al, 1973; Bailey et al, 1976). Moreover, peripheral blood lymphocytes from patients with cirrhosis and acute alcoholic hepatitis were found to be cytotoxic to isolated rabbit hepatocytes and a close correlation was found between the presence of

cytotoxic lymphocytes and portal and periportal imflammation (Cochrane et al, 1977).

Peripheral T lymphocytes were reduced in patients with alcoholic hepatitis (Berenyl et al, 1974), and acetaldehyde caused inhibition of leukocyte migration in alcoholics with liver disease (Actis et al, 1978).

B. METHODS USED FOR INDUCING EXPERIMENTAL LIVER CIRRHOSIS

Proper models for the development of liver cirrhosis in experimental animals are essential for studying diagnostic, preventive and therapeutic measures.

Experimental evidence suggests that a number of chemical compounds can alter the course of postinflammatory fibrosis and subsequent cirrhosis. Therefore, a good animal model for studying the pathogenesis of liver cirrhosis is mandatory.

Most commonly used models are induction by carbon tetrachloride (CCl_4), thioacetamide, dimethylnitrosamine (DMNA), alcohol and nutritional manipulation.

1. Carbon tetrachloride (CCl_4).

CCl_4 is a hepatotoxic agent used in the past as a carpet cleaner and spot remover. It has also been used for treatment of hookworm infestations (Recknagel, 1967). Cameron and Karunaratne (1936) induced experimental liver cirrhosis in rats by the administration of CCl_4 in a dose of 0.1 ml twice a week for six months. Islami et al (1958) induced advanced cirrhosis in rats by 45 injections of CCl_4) over 90 days. Rubin et al (1963) showed that all

experimental animals which were subjected to CCl_4 developed cirrhosis. They also found that connective tissue increased progressively between 20 and 65 days of CCl_4 administration.

Maros et al (1975) found that fibrosis was irreversible after 4 months of CCl_4 administration, while fibrosis was reversible if CCl_4 was discontinued within 3-4 months. Motfort and Perez-Tamayo (1978) observed that collagenase was present in connective tissue septa as long as cirrhosis was reversible, while it was not detected in the irreversible stage. Toh et al (1977) observed an increased expression of actin-like protein in regenerating hepatocytes and granulation tissue fibroblasts in cirrhotic livers.

Chronic CCl_4 administration in rats produces histologic and biochemical changes similar to those seen in humans (Popper, 1977; Leevy et al, 1976; McLean and McLean, 1969).

Mechanism of action: CCl_4 is activated to CCl_3 (trichlormethyl radical) which is covalently attached to lipids and proteins causing lipid peroxidation (fig.2).

EFFECT OF $CC1_4$ ON THE LIVER CELL

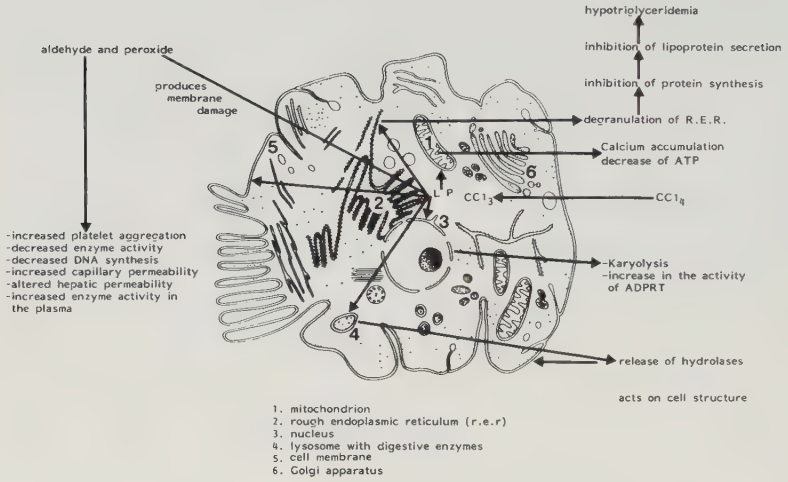


Fig.2. The mechanism of action of CCl_4 on the liver cell.

Lipid peroxidation inhibits activities of many enzymes, increases capillary permeability and causes platelet aggretation (Slater, 1978; Glende et al, 1976; Recknagel and Glende, 1973). Administration of CCl_4 rapidly damages endoplasmic reticulum and thereby depresses protein synthesis to a significant degree (Recknagel and Glende, 1973).

Within few minutes of CCl_4 administration, vacuolization and

degranulation of components of endoplasmic reticulum, loss of glucose-6-phosphatase activity, intracellular accumulation of calcium and alterations of various cytoplasmic components of the liver cell are observed (Reynolds, 1963). A few hours later there is a 30 percent decrease in the liver's content of NADPH (Slater, 1978). CCl_4 increases lipid deposition, causes decreases in the cellular level of adenosine triphosphate (ATP), modifies the mitochondrial function, and reduces the rate of amino acid utilization (Recknagel and Litteria, 1960; Dianzani et al, 1966; Wands et al, 1970; Smuckler et al, 1968; Smuckler, 1966). Impairment of protein synthesis and dislocation of polyribosomes are noticed between 15-30 minutes of CCl_4 administration (Gravela and Dianzani, 1970; Smuckler and Benditt, 1965).

Cameron and Karunaratne in 1936 showed the development of liver cirrhosis in rats after subcutaneous administration of CCl_4. From that time a number of investigators have tried this hepatotoxin to induce liver cirrhosis by using different routes of administration. The major problem in these studies have been a high mortality rate associated with a poor reproducibility. One factor responsible for this lack of reproducibility is the inconsistent response of the rat liver to CCl_4. This makes the same dose lethal to one rat while being insufficient to inflict damage in another.

In 1982 Proctor and Chatamra reported a method of producing liver cirrhosis in rats by intragastric administration of CCl_4 with prior treatment of the animals with phenobarbitone. In our hands a 27% mortality rate was obtained with this model and a development of cirrhosis in about 75% of surviving animals. These results are in agreement with results obtained by Proctor and Chatamra.

The production of cirrhosis requires a succession of liver injuries alternating with periods of repair. Both the intensity of the injury and the time allowed for recovery are important. Trials of different routes of administration with or without adjuvant substances have not been performed. The use of young animals should also be explored, since they have fully developed mixed-function oxidase system, necessary for the deletarious action of CCl (McLean, 1966; Slater, 1978; Trivedi, 1983).

A limited number of studies were carried out to investigate the role of trace elements in the pathogenesis of liver cirrhosis. Since zinc is involved in many body functions such as immunity, wound healing, growth and carbohydrate and protein metabolism, zinc has been tried for therapeutic, preventive and diagnostic measures in liver cirrhosis.

It was found that zinc ions have a protective effect against

liver injury. Ludwig and Chvapil (1982) found that pretreatment of rats with 2 mg of zinc chloride per 100 g of body weight intraperitoneally for three consecutive days resulted in 50 percent reduction of CCl_4 -induced centrilobular necrosis along with significant reduction of the lysosomal enzyme β-Gluc in zinc treated animals. Similar results were obtained by Cagen and Klassen (1980). They observed a marked reduction in both ALAT and ASAT along with a marked elevation of hepatic concentration of metallothionein in animals pretreated with zinc chloride. They suggested that zinc-induced metallothionein may play a role in zinc-mediated protection against CCl_4 toxicity.

Yunice and Lindeman (1977) found that pretreatment of mice with zinc and ascorbic acid prior to administration of toxic doses resulted in significantly better survival than controls. Anttinen et al (1984) suggested that peroral zinc treatment has a direct and selective inhibitory effect on carbon tetrachloride-induced collagen accumulation in rat liver. It is not known if chronic zinc administration can modify the development of cirrhosis in experimental animal.

Furthermore, it was demonstrated that short-term oral supplementation of zinc (600 mg of zinc acetate a day for 7 days) improved patients with chronic hepatic encephalopathy and increased urea synthesis (Reding et al, 1984). It is not

known what involvement zinc has in the pathogenesis of encephalopathy, verified more elaborately than by trailmaking test alone as in this study.

2. Thioacetamide.

Thioacetamide was originally used for the control of orange decay (Childs and Siegler, 1946). It is a white crystalline powder, easily dissolved in alcohol and water. It undergoes hydrolysis and liberates H_2S. Thioacetamide has been recommended for the induction of experimental liver cirrhosis because of its rapid elimination and cumulative injury when it is given intermittently (Brodehl, 1966). Thioacetamide is effective in the production of liver necrosis, cirrhosis, bile duct proliferation and liver tumours (Hunter et al, 1977; Fitzhugh and Nilson, 1948; Gupta, 1956; Muller et al, 1982; Dashti et al, 1985; Dashti et al, 1986). Nygaard et al (1954) found that the thyroid, adrenal glands and the bone marrow in rats were able to concentrate thioacetamide without being affected as compared to the liver.

Mechanism of action: Thioacetamide metabolised by cytochrome P-450 and thioacetamide-s-oxide metabolism may lead to cellular damage (Hunter et al, 1977; Porter and Neal, 1978;

Porter et al, 1979) (fig.3.).

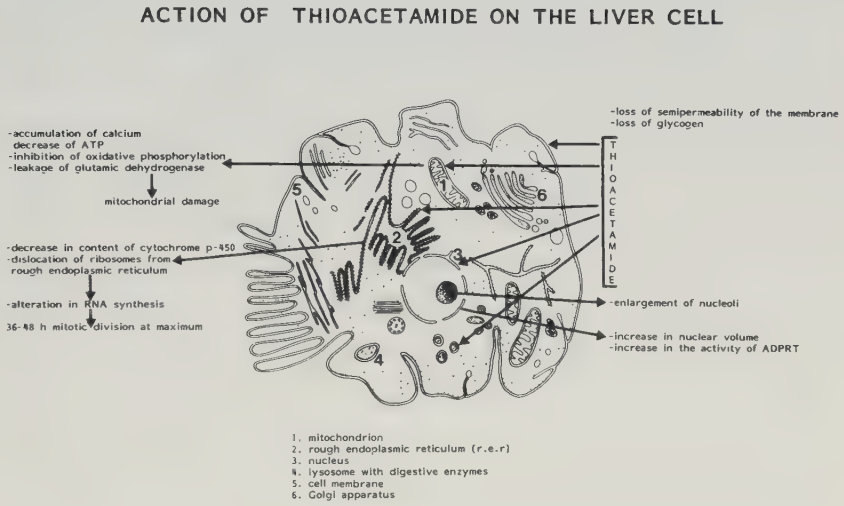


Fig.3. Mechanism of action of thioacetamide on the liver cell.

Gallagher et al (1956) observed a rise in liver calcium and a fall in liver magnesium. They postulated that thioacetamide inhibits respiratory metabolism of the liver and that this inhibition is mainly due to accumulation of calcium in the cell. Barker et al (1963) showed that thioacetamide decreases protein synthesis in the liver and pancreas by its dislocation of ribosomes from endoplasmic reticulum. An increase in DNA synthesis and mitotic activity

between 36 and 48 h after a single injection of thioacetamide have been shown in rats (Reddy et al, 1969). These authors suggested that the cellular proliferation could be due to the effect of thioacetamide on the nucleolus. An increase in the nucleolar size and in the RNA synthesis in liver cells after thioacetamide administration have been reported by many investigators (Kizer et al, 1965; Kleinfield, 1966; Rather, 1955; Steele and Bush, 1966; Steele et al, 1965; Fausto, 1970). Fausto (1970) observed an increase in decarboxylase activity along with accumulation of RNA in the nucleus after a single injection of thiocacetamide in rats. Morley and Boyer (1977) found that administration of low doses of thioacetamide stimulated DNA synthesis, and they detected growth factors in the serum of the rat within 6 h of thioacetamide administration. Hunter et al (1977) observed that thioacetamide sulfine, which is a metabolic product of thioacetamide, was responsible for the hepatotoxicity via inhibition on the activity of hepatic mixed function oxidase system. Younes et al (1983) have shown that thioacetamide destroys microsomal calcium-pump activity in in-vitro studies. Furthermore, they observed failure of thioacetamide to release lysosomal enzymes. However, in our in-vivo studies using thioacetamide, we found only small releases of the lysosomal enzymes β-NAG and β-Gluc after thioacetamide administration, except when higher doses were used. Ono et al (1973) showed that the

activity of hepatic ornithine decarboxylase increased following the administration of thioacetamide. The activity of this enzyme was also increased following the administration of growth hormone to normal animals. It has been shown that thioacetamide induces morphological changes in the liver resembling changes seen after partial hepatectomy (Rather, 1951).

Most previous studies have been performed in rats with intraperitoneal or subcutaneous administration of thioacetamide (Gallagher, 1956; Gupta, 1956). A few studies have been performed by mixing the compound with the diet (Rather, 1951; Fitzhugh and Nilson, 1948; Gupta, 1956). Administration of thioacetamide in drinking water has not been studied much. Müller et al (1982) have shown that thioacetamide in a dose of 0.3 g/l in drinking water produced liver cirrhosis within 2.5-3 months in female Wistar rats, although the weight of the animals was not given. We found that the administration of 0.3 g/l to male Wistar rats weighing 150-160 g induced liver cirrhosis in 75% of the animals with no mortality and was an efficient way of inducing liver cirrhosis and superior to that of CCl_4-administration. Since the extent of liver cell damage induced by chronic administration of thioacetamide differs to a considerable degree, the effect of only one dose of thioacetamide on biochemical parameters, trace elements and

histology would be of interest to study.

3. Dimethylnitrosamine (DMNA).

DMNA is another hepatotoxin which produces changes in the liver similar to those seen in human liver cirrhosis when administered to dogs and rats (Maddan et al, 1970; Testam et al, 1978). The animals not only develop portal hypertension and biochemical changes, e.g. increased retention of BSP and altered liver function tests, but also necrosis and nodular regeneration. Administration of DMNA in a dose of 50 mg/kg body weight intragastrically once a week to rats induced hepatic cirrhosis after 12 weeks with no evidence of tumour formation in one study. Death occurred after 17-23 weeks of administration (Steinhoff, 1975). Kohno et al (1977) induced liver cirrhosis in dogs by constriction of hepatic vein and administration of DMNA.

The disadvantages of this hepatotoxin are the frequent incidence of hepatoma along with cirrhosis, the long duration of induction and the high mortality.

4. ALCOHOL.

Although alcohol is the main toxin behind human liver cirrhosis, the long exposition time needed do not allow production of cirrhosis by alcohol in rodents. Addition of alcohol to drinking water induces fatty livers to 100% of baboons. The histologic and biochemical pattern resembled the human variety in all aspects (Goldsmith and Moor-Jankowski, 1972). Alcohol has been regarded as a direct hepatotoxin (Rubin and Lieber, 1975). Of 15 ethanol fed baboons, all developed fatty liver, five progressed to hepatitis and five had cirrhosis and the lesions were similar to those observed in human alcoholics (Lieber et al, 1975). Wallerstedt et al (1975) fed guinea-pigs with 40 percent of calories as ethanol for 8 months. In this study cholesterol and triglycerides increased in the liver and serum and a slight depression of the coagulation factors II, VII, X and XI was observed in the ethanol-fed animals. In baboons fed ethanol perivenular fibrosis was associated with myofibroblast proliferation followed by collagen deposition (Nakano and Lieber, 1982).

Hepatic lesions similar to those induced by ethanol in humans and baboons can not be produced in rats as the life expectancy of rats is around two years, furthermore, alcohol has not as grave action as other hepatotoxins.

Combination of other hepatotoxins with alcohol may enhance the development of liver cirrhosis in rodents, but this has not been studied extensively.

5. Nutritional manipulation.

Cirrhosis is seen in patients with severe malnutrition such as Kwashiorkor disease and malignant protein-calorie malnutrition (Ramlingaswami, 1964). Therefore, the production of experimental liver cirrhosis has been tried by feeding animals diets deficient in different nutrients (Bengmark et al, 1966). Changes of the dietary intake (both excess and deficiency) have also been used to induce cirrhosis in experimental animals.

5.1. High cholesterol diet.

Hepatic changes were induced in rabbits fed on different regimens of high cholesterol diet for a prolonged period of time (Gupta et al, 1976). The liver exhibited fatty changes. There was also initially centrilobular and later diffuse fibrosis, which eventually developed into portal cirrhosis of monolobular type.

5.2. Hypolipotropic diet.

The diet is composed of 8 percent casein, 48 percent sucrose, 38 percent lard, salts, vitamins and cysteine along with 0.4 percent choline chloride. This diet was given to rats for a period of 4-30 weeks. 10 percent of the rats died of haemorrhagic renal necrosis and the rest developed fatty changes of the liver and liver cirrhosis (Ohta, 1963).

5.3. Choline deficiency.

Rhesus monkeys were fed a choline and protein deficient diet for a period of 31 months. After 26 months most of the animals developed cirrhosis (Patek et al, 1975). Bengmark and associates (1966) induced cirrhosis in rats by feeding a choline deficient diet for 8 months.

These models of nutritional cirrhosis have not been used widely. Several reasons for this can be found. The induction time is prolonged, which makes them expensive and cumbersome. It also makes it necessary to perform studies in only older animals. The dietary manipulation may induce alterations in other organs besides the liver and metabolic processes which may affect secondarily the intended studies.

Through the land I wandered alone,
the land where the shadows spread
Far out in the desert, 'mongst thistle and bone,
I spoke with the dead.

From The Pilgrim's Christmas Song,
by Verner von Heidenstam (1859-1940)
Nobel Prize, 1916

C. AIMS OF THE PRESENT STUDY

The present investigation has been designed to increase the understanding of factors involved in the pathogenesis of liver injury and cirrhosis. The definition of a simple and reproducible model of liver cirrhosis in experimental animals in relation to biochemical (transaminases and lysosomal enzymes) and histological changes is essential. Profound alterations of trace elements, especially of zinc and copper are seen in different liver diseases in humans, and therefore, the possible relationship of these changes to the development of liver cirrhosis is important. Finally the suggestions of a role of oxygen free radicals in liver injury are of importance for a possible preventive and therapeutic measure in liver cirrhosis.

The studies were performed:

1. to study the early biochemical and histological changes after thioacetamide exposition to rats;
2. to study the oral administration of thioacetamide for

producing liver cirrhosis, its comparison to CCl_4-induction and associated changes of trace elements;

3. to study the reversibility of experimental liver cirrhosis;
4. to study a simple way of inducing experimental liver cirrhosis;
5. to study repeated liver resections in cirrhotic animals and associated changes of trace elements;
6. to study zinc administration during development of cirrhosis;
7. to study alterations of zinc in human alcoholic cirrhosis and their role for encephalopathy; and
8. to study oxygen free radicals in toxic liver cell injury and the prevention by scavenger.

It was evening in the mountains.
The air was like a cold goblet,
a dry transparency.
I had lived on many vessels before,
but in the desert night
the enormous mine shimmered
like a sparkling vessel
with the glittering dew
on the darkened hilltops.

From The Lamp on the Ground
by Pablo Neruda (1904-1973)
Nobel Prize, 1971

D. METHODOLOGICAL CONSIDERATIONS

1. Subjects.

1.1. Rats.

Male Wistar rats from Anticimex, Sweden, were used in all experiments. All rats were fed standard food pellets (Brood Stock Feed for Rats and Mice-R3, Evos-Anticimex, Södertälje, Sweden) and water ad libitum. The rats in paper IV had an initial weight of 60-80 g, in paper I, III and VI 150-180 g, in paper IX 90-120 g, in paper V 180-200 g and in paper II 450-490 g. The animals were housed 3 per cage and the light was on between 7 a.m. and 7 p.m..

1.2. Patients.

1.2.1. Human study (paper VII). 22 patients with stable liver disease and without any medical treatment during the

study participated in the study. They had biopsy-proven alcohol-induced cirrhosis and a history of bleeding from oesophageal varices and encephalopathy.

1.2.2. In vitro study (paper VIII). Venous blood was collected from apparently healthy human donors without known history of any disease or alcohol abuse.

2. Experimental models.

2.1. Thioacetamide administration.

2.1.1. Subcutaneous administration of thioacetamide. Thioacetamide (Sigma) was dissolved in deionized water (1 ml) and was administered in a dose of 200 mg/kg body weight subcutaneously. Control animals were injected with an equal quantity of deionized water (1 ml) subcutaneously. At sacrifice blood was drawn from the abdominal aorta under light ether anaesthesia in metal free tubes and plasma was separated. The liver was removed, weighed and a small part was kept in buffered formalin for histopathological assessment. Approximately 1 gm of the liver was kept frozen for trace element analysis.

2.1.2. Oral administration of thioacetamide (II, III).

Animals were either given thioacetamide in a dose of 0.5 g/l in the drinking water for a period of 12 weeks (II), or 0.3 g/l and groups of animals sacrified after 2, 4, 6, 8 and 16 weeks under light ether anaesthesia (III).

2.2. Carbon tetrachloride administration.

2.2.1. Intragastric administration of CCl_4 (III). Rats were pretreated for 14 days with phenobarbital mixed in the drinking water at a concentration of 350 mg/l before CCl_4 administration. Thereafter the animals were given CCl_4 intragastrically once a week under light ether anaesthesia according to Proctor and Chatamra (1982). The initial dose was calculated and found to be 0.08 ml of CCl_4. Thereafter the dose was adjusted weekly after changes of body weight. Animals were sacrified at the end of 16 weeks.

2.2.2. Intraperitoneal administration of CCl_4 mixed with olive oil. 0.15 ml of CCl_4 mixed with olive oil (1:7, v/v) was administered 3 times a week for 7 weeks (IV). CCl_4 was administered in a dose of 1 ml/kg B.W. mixed with olive oil in a ratio of 1:5 (v/v) (XI).

2.2.3. Administration of CCl_4 with alcohol (VI). 0.2 ml CCl_4 mixed with olive oil (1:9, v/v) was administered intragastrically twice a week for a period of 8 weeks

(Heitmann et al, 1982). During the same period animals were drinking water mixed with alcohol in a concentration of 10 percent.

2.3. Liver resection (V). Cirrhotic rats were subjected to liver resections under ether anaesthesia using clean but not sterile technique. The abdomen was opened via a midline incision. The liver was mobilized and the medial lobe (about 35% of liver weight according to own unpublished observations) was ligated and removed. Some animals were resected 6 weeks later, at what time the right anterior lobe was removed (about 40% of the liver weight). Surviving animals were sacrified 6 weeks after the second resection.

2.4. Administration of zinc. Zinc sulphate (Kebo, Stockholm) was dissolved in deionised water and administered in a dose of 50 mg/kg B.W. for three days and 100 mg/kg B.W. one hour prior to the administration of thioacetamide (I). In another study (VI) zinc was prepared in the same way and administered intragastrically three times a week in a dose of 25 mg/kg B.W. simultaneously with CCl_4.

2.5. Administration of SOD. Superoxide dismutase (Boehringer, F.R.G.) was dissolved in distilled water and administered intraperitoneally in a dose of 10.000 U 1 h before, simultaneous with and 1 h after CCl_4 administration

(IX). SOD was also used in the in-vitro study (see below).

3. Psychometric tests (VII). The psychometric tests were performed in the morning under equivalent conditions by the psychologist. The psychometric tests utilized in the study are in general use at the Department of Psychiatry, Lund University, Lund, in diagnosing diffuse organic brain dysfuntions. The tests were carried out individually by each patient within one h. The test battery was designed to evaluate both perceptual and psycho-motor functions. Lowered performance of these tests reflects inattentiveness and a slowing of general response, typical of diffuse brain dysfunction and toxic encephalopathy (Willanger, 1970; Lezek, 1976; Hagberg, 1978). The following tests were used:

Synonyms (SRB:1): A timed test assessing verbal ability and providing an estimate of the premorbid intellectual capacity. The patient is asked to choose one of five words as synonymous to a key-word. A high score indicates a good performance.

Logic inductive test (SRB:2): Among groups of five geometrical designs presented, four are alike in some way. The patient is instructed to identify the one that differs. A high score indicates a good performance.

Block Design (SRB:3): Measures visual spatial performance as well as logic inductive capacity. The present version is based on Koh's block design. A high score indicates a good performance.

Memory For Design (MFD): Assesses the spatial perceptive ability and the immediate memory for simple geometric designs. The score analysis is presented in terms of error points. A low score indicates a good performance.

Visual Retention Test (Benton): Tests conceptual memory, visual perception abilities and memory for geometrical forms. The designs consist of more but simpler figures than the MFD test and stress the memory capacity. BENR: The total score is related to number of rights. A high score indicates a good performance. BENW: The score is related to number of wrongs. A low score indicates a good performance.

Word Pair Test (WP): Consists of three subtests with ten paired associates in each. One paired associate per second is presented, ten seconds later the first word in each pair is given and the subject is supposed to recall the second. The test is sensitive for memory dysfunctions. A high score indicates a good performance.

Digit Symbol Test (DIG): A visual motor test that is a

subtest of WAIS. The patient is given a list of symbols associated with digits from 1 to 9 and is asked to fill in blanks with symbols that correspond to each number. A high score indicates a good performance.

Dot Test: A modified version of the Bourdon-Wirsma vigilance test for perceptual speed. The instrument presents a task of eliminating groups of dots of specified character contained in rows of similar but different stimuli. The performance is highly dependant on perceptual speed (DOTT) and accuracy (DOTW) and sensitive to concentration difficulties, lack of tenacity and to fatigue (DOTF). Low score indicates good performance.

Colour Word Test (CWT): A version of the Stroup Colour Word Test. It is used to assess cognitive flexibility. First the subject is asked to name the colour of 100 triplets of the symbol x and then read the printing colour of 100 colour-words where the printing colour is incongruent to the word. The time for reading the second part is measured. A low score indicates a good performance.

Manual Dexterity (CYL): The test consists of lifting cylindrical knobs from one board and placing them in corresponding holes in another identical board. The number of knobs correctly placed with the subject using the

dominant hand is recorded. A high score indicates a good performance.

Trailmaking Test: Version A and B: These tests measure cognitive motor abilities. This version consists of connecting numbers and letters arranged in a circle requiring the patient to deal with two dimensions. The combined score (TMT) and the score difference (TMD) between the two versions are given. A low score indicates a good performance.

Reference Material: Fifty apparently healthy industrial workers without known alcohol abuse and normal liver tests served as reference subjects for the psychometric test results. Obviously there is no sharp limit between normal and abnormal cerebral function. However, we decided to use the commonly accepted mean 2SD to calculate the reference normal limits of the test scores. The use of this method is dependant on normal distribution which was tested according to Filliben (1975). The scores of five psychometric test (MFD, BENW, DOTW, DOTF and TMD) were not normally distributed and transformed by applying the square root to fulfill the requirements of normal distribution.

4. Preparation of cell suspensions (VIII, IX).

Venous blood was collected from apparently healthy human donors in vacutainers containing heparin (143 USP units/tube). The platelet rich plasma was removed by centrifugation at 100 x g for 10 minutes. The platelets were removed from the plasma by centrifugating at 400 x g for 25 minutes in order to prepare platelet poor plasma (PPP). The cell suspension was diluted to its original volume with physiologic saline and then layered onto Isopaque-Ficoll density cushion (Nyregaard) and spun at 400 x g for 25 minutes. The mononuclear leukocytes were isolated from the interphase zone of the gradient. The cells were washed in saline and counted, then readjusted and resuspended at 0.5 x 10^6 cells/ml in 4 ml Eagle's medium with Hank's salts (Gibco) plus 1 ml of autologous platelet poor plasma. To the cell suspensions were then added various concentrations of ethanol (96%), carbon tetrachloride and thioacetamide (Sigma). The final concentrations in the suspensions ranged from 50 µl (1mM) to 150 µl (3mM) for ethanol, 0.1mM to 1 mM for CCl_4 and 0.1mM to 10 mM for thioacetamide. CCl_4 was dissolved in alcohol so that a total volume of 50 µl of this solution was given to the cell suspensions. Thioacetamide was dissolved in physiologic saline and then adjusted so that 50 µl aliquots were added to the cell suspensions. The superoxide scavanger, superoxide dismutase (SOD)

(Boehringer, F.R.G.), was added to half of the suspensions. SOD was dissolved in physiologic saline and 50 µl of SOD containing 200 µg of SOD was added to the cell suspensions. All cell suspensions together with controls were made in duplicates and incubated for 1 h at 37 ^{o}C.

5. Extraction and analytic procedures.

5.1. Trace elements analysis.

All plasma samples were collected in special metal free tubes (vacutainer, Becton and Dickinson, U.K.). Plasma was separated within 4 h of sampling using acid washed Pasteur pipettes (Labora, Stockholm, Sweden) and stored at -20 ^{o}C pending analysis. Plasma samples were diluted with double distilled water. Accuracy of the analysis was checked using autonorm plasma (Nyregard, Diagnostica, Norway) as a reference material. All analysis were performed by atomic absorption spectrophotometry (Varian AA6).

The liver samples were wet-ashed using concentrated sulphuric, nitric and perchloric acid. The wet-ashing was performed in 50 ml Kjeldhal flasks. 0.5 g of liver tissue was used for wet ashing. 1 ml of concentrated sulphuric acid and 3 ml of a concentrated perchloric-nitric acid mixture were added to the liver samples. The procedure was

continued by adding 1 ml of aliquots of the nitric-perchloric acid mixture until a slight charring occurred. The temperature during the ashing process was kept around 180 ^{o}C. The excess of nitric acid was boiled out and the content of the Kjeldhal flasks was mixed with 5 ml of deionised water and analysis for zinc and copper was performed by atomic absorption spectrophotometry (Varian AA6).

5.2. Liver function test.

ALP (alkaline phosphatase), ASAT (aspartate aminotransferase), ALAT (alanine aminotransferase) were determined according to the methods of the Committee on Enzymes of the Scandinavian Society of Clinical Chemistry and Clinical Physiology (1974, 1981). Bilirubin was determined by the method of Küffer et al, (1974).

5.3. Lysosomal enzymes.

β-glucuronidase (β-Gluc).

100 µl 1mM 4-methylumbelliferyl-β-D- glucuronide (Koch-Light Ltd, Colnbrook, U.K.) in 100 mM citrate-phosphate buffer, pH 4.5 and 25 µl plasma were incubated at 37 ^{o}C for 30 min. The reaction was stopped with 3 ml of 0.2 M glycine buffer, pH 10.7 and fluorescence was read in a spectrofluorimeter with an excitation wavelength of 365 nm and an emission wavelength of 478 nm. 4-Methylumbelliferone

in glycine buffer was used as standard (Hultberg et al, 1983).

β-hexosaminidase (β-NAG).

50 µl 7.5 mM p-nitrophenyl-2-acetamide deoxy-β-D-glucopyranoside (Koch Light Labs Ltd) and 50 µl 1 M citrate buffer, pH 4.5 and 100 µl plasma were incubated at 37 ^{o}C for 30 min. The reaction was stopped with 3 ml of 0.2 M glycine buffer, pH 10.7, and read within 30 min in a spectrophotometer at 400 nm. p-Nitrophenol was used as a standard.

For both the amount of enzyme was defined as mol substrate split per min (U)per liter plasma (Hultberg et al, 1983).

5.4. Measurement of adenosine diphosphate ribosyl transferase (ADPRT) activity.

After the incubation period the mononuclear leukocytes were harvested by centrifugation at 300 x g for 10 minutes. ADPRT was estimated according to procedure of Berger (1978), with modifications by Pero et al (1983). Briefly, the cells were permeabilized on ice in hypotonic buffer (0.01 M Tris-HCl, pH 7.8; 1mM EDTA; 30 mM 2 mercaptoethanol; 4mM $MgCl_2$) and the cytosol removed by centrifugation of the cytoskeleton. Next ^{3}H labeled NAD^{+} (155 µ M, 150 mCi/mmol, ^{3}H-label in adenine) was added in reaction buffer (100 mM Tris-HCl, pH 7.8; 120 mM $MgCl_2$). The ADPRT

activity was recorded as the TCA precipitable cpm ^{3}H-NAD^{+} incorporated per million cells. In study IX the ADPRT activity was recorded as the TCA precipitable cpm ^{3}H-NAD^{+} per mononuclear leukocytes present in 1 ml of whole blood.

5.5. Histological assessment.

Liver tissue was fixed in buffered formalin, embedded in paraffin and sections were stained with haematoxylin-erythrosine (htx-e). In study IV, V and VI the specimens were stained with reticulin (Gordon Sweet's variant) and collagen stains (van Gieson).

6. Statistical evaluation.

Student's t-test was used in all experimental studies (I, II, III, IV, V, VI, VIII and IX).

In study VII Mann-Whitney rank sum test was applied to compare patient populations, that were not normally distributed. Pearson product moment correlation was used for multiple correlation analysis.

And from The Tree of Knowledge I ate of the fruit with you,
and on winding paths I crossed the desert.

The Song of the Pearl,
by Czeslaw Milosz (1911)
Nobel Prize, 1980

E. RESULTS AND DISCUSSION

1. Experimental models of liver cirrhosis (II, III, IV and V).

There was no mortality in the animals exposed to thioacetamide in a dose of 0.3 g/l in drinking water. There was a significant reduction in body weight compared to controls. One of eight animals showed liver cirrhosis at the end of 8 weeks; at the end of 16 weeks 6 of 8 animals showed cirrhosis. After 2 weeks of thioacetamide intake, histopathological studies of the rat liver showed an increased eosinophilic staining of hepatocytes around central veins. These cells had large nuclei with a large central nucleolus. There was also an increased number of Kupffer cells centrilobularly (fig.4.).

After 4 weeks lymphocytes and neutrophils were gathered in the central regions and some hepatocytes in this region were necrotic. Hypertrophy of nuclei and nucleoli was observed in all regions of the lobules, later on macrophages with yellow pigments appeared around the

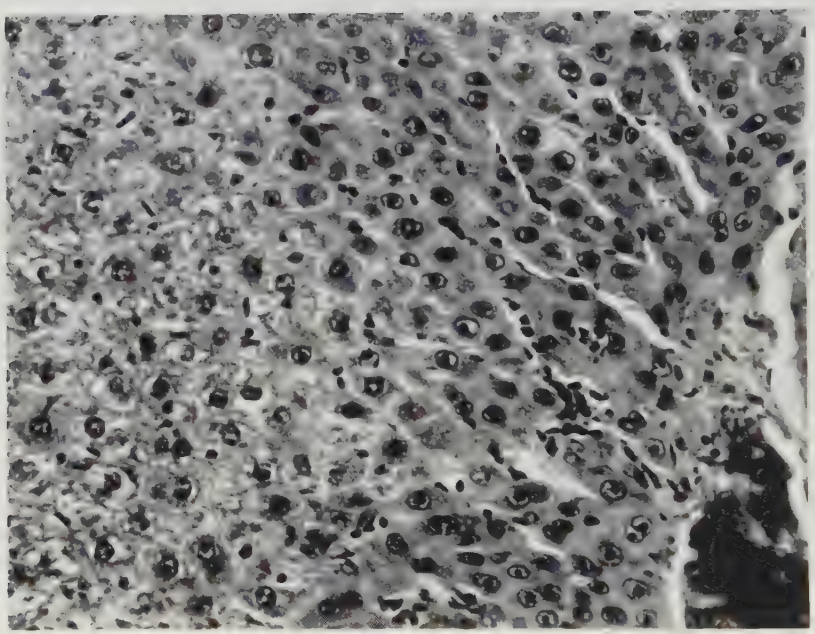


Fig.4. Rat liver after 2 weeks administration of thioacetamide. There are increased numbers of Kupffer cells in the centrilobular region near the central vein to the right. There are also large hepatocytic nuclei with prominent nucleoli and increased staining of the cytoplasm of the hepatocytes (htx-e /325).

central veins, probably secondary to phagocytosis of necrotic hepatocytes. Thereafter fibrous strands appeared in the central regions, and these strands later on connected central veins with portal tracts (at 8 weeks) (fig.5.)

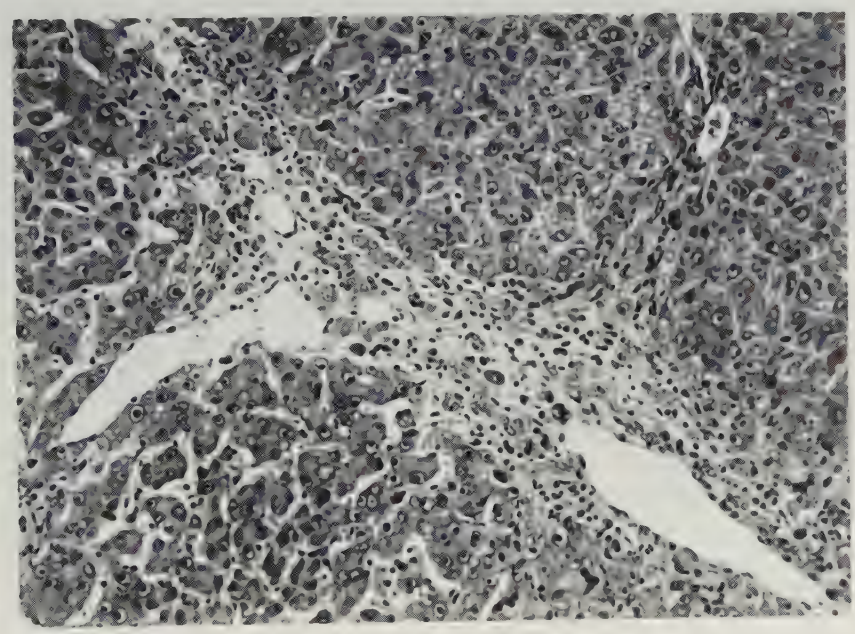


Fig.5. Rat liver after 8 weeks administration of thioacetamide. There are two central veins in the central part and a small portal tract in the upper right. These structures are connected by connective tissue with inflammatory cells (htx-e /200).

At the same time abnormal round nodules of hepatocytes were observed. At 16 weeks some rats showed diffuse cirrhosis with varying degree of cellular pleomorphism in different regenerating nodules. No tumour transformation was noted macro- or microscopically (fig.6).

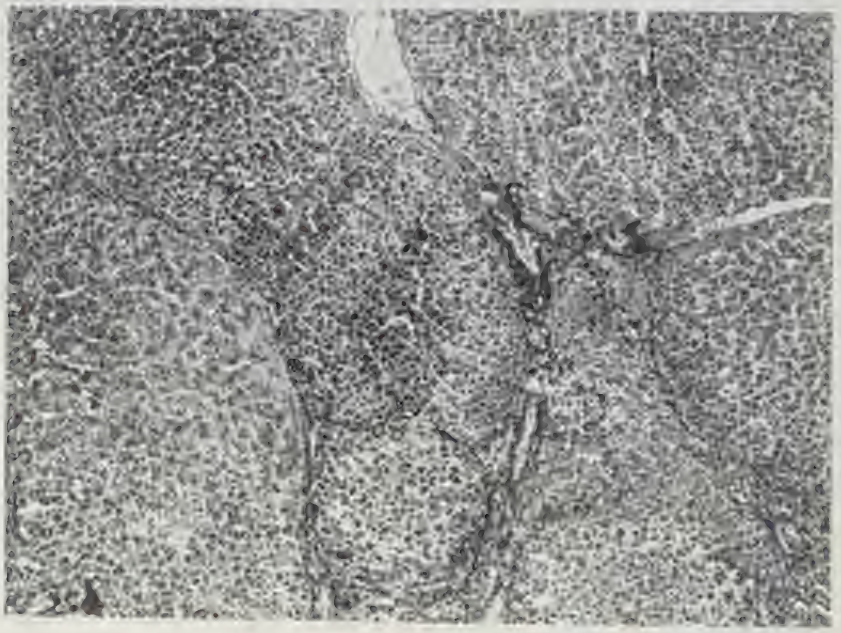


Fig.6. Rat liver after 16 weeks administration of thioacetamide. Note the more marked regeneration nodules (htx-e /85).

In the group of rats receiving CCl_4 according to Proctor and Chatamra 8 out of 11 animals survived at 16 weeks (27% mortality). 6 animals showed cirrhosis (75% of surviving animals) (fig.7).

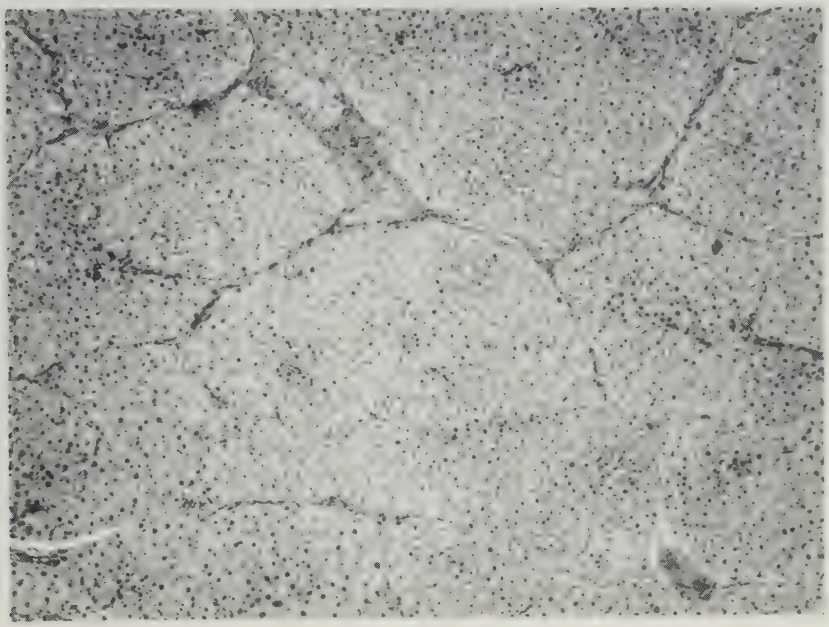


Fig.7. Liver cirrhosis after intragastric administration of CCl_4 (htx-e /85).

Macroscopically the liver surface was rather smooth. There were small nodules of uniform size. In thioacetamide-rats (16 weeks) the liver had a nodular surface and exhibited nodules of different sizes. Microscopically CCl_4 cirrhosis showed regular hepatocytes, while the thioacetamide cirrhosis had pleomorphic hepatocytes.

Biochemical changes: The plasma level of β-Gluc decreased significantly after 16 weeks, while β-NAG was slightly decreased only at 8 weeks (table 1).

Table 1

ASAT, ALAT, β-NAG and β-Gluc in plasma of experimental and control rats

	ASAT(ukat/l)	ALAT(ukat/l)	β-NAG(U/l)	β-Gluc(U/l)
CCl_4(16 weeks)	4.3± 3.9	2.2 ±1.0		
Thioacetamide				
Controls (n=8)	1.5 ±0.25	0.9 ±0.2	13.2 ±3.3	0.44 ±0.14
2 weeks (n=8)	2.2 ±0.56*	1.0 ±0.17	15.0 ±1.5	0.55 ±0.1
4 weeks (n=8)	1.4 ±0.23	0.6 ±0.11*	22.7 ±1.1**	0.48 ±0.1
6 weeks (n=8)	1.95 ±0.5	0.61 ±0.12*	13.4 ±1.9	0.45 ±0.06
8 weeks (n=8)	1.51 ±0.26	0.5 ±0.15**	8.7 ±1.5*	0.35 ±0.03
16 weeks (n=8)	1.4 ±0.36	0.45 ±0.05**	12.7 ±0.8	0.17 ±0.09**

*denotes $p < 0.025$ compared to control animals

**denotes $p < 0.005$ compared to control animals

The level of ASAT was significantly increased after 2 weeks, afterwards there was no significant change, while ALAT decreased significantly at 4, 6, 8 and 16 weeks. In the group of rats receiving CCl_4 ASAT and ALAT increased significantly at 16 weeks.

We found in this experiment that the administration of thioacetamide in a dose of 0.3 g/l to animals of 150-160 g induced liver cirrhosis in 75% of the animals with no mortality and was an efficient way of inducing liver cirrhosis in rats and superior to that of CCl_4 -administration.

There was an early appearance of centrilobular necrosis in the thioacetamide group followed by inflammation and fibrosis. Thioacetamide also induced more nuclear atypia and mitosis than CCl_4. These differences may indicate a different injurious action of the two hepatotoxins. It is well established that CCl_4 increases the fragility of biological membranes and leads to an increased lipid peroxidation and lysosomal damage (Recknagel and Ghoshal, 1966; Glende et al, 1976; Slater, 1978; Younes et al, 1983; Melen et al, 1986).

The administration of thioacetamide decreases the hepatic content of P-450, inhibits the respiratory metabolism and

enzymatic activity within the nucleus of liver cells, it also increases DNA synthesis and mitosis of hepatocytes (Barker and Smuckler, 1972; Gallagher et al, 1956; Rather, 1951; Reddy et al, 1969). This indicates that there may be different mechanisms of action of thioacetamide and CCl_4.

Both hepatotoxins induced an increase of ASAT, although thioacetamide to a smaller extent. Thioacetamide administration led to a transient decrease of plasma β-NAG and a decrease in β-Gluc. Similar findings have been described by Weber et al (1983). CCl_4 administration has also been shown to cause increased levels of β-NAG (Melen et al, 1985). It has been suggested that macrophages activated by gut antigen bypassing the filtering action in the liver are the cellular source for the increase of serum lysosomal enzymes (Isaksson, 1985).

In order to study if alteration of the dose of thioacetamide, could reduce the induction time, we studied administration of thioacetamide in a dose of 0.5 g/l in a similar way as above (II). The animals receiving this dose lost weight to a considerable extent compared to control animals. Cirrhosis was found in all rats at sacrifice after 12 weeks. The cirrhosis was of mixed macro- and micronodular type (fig.8).

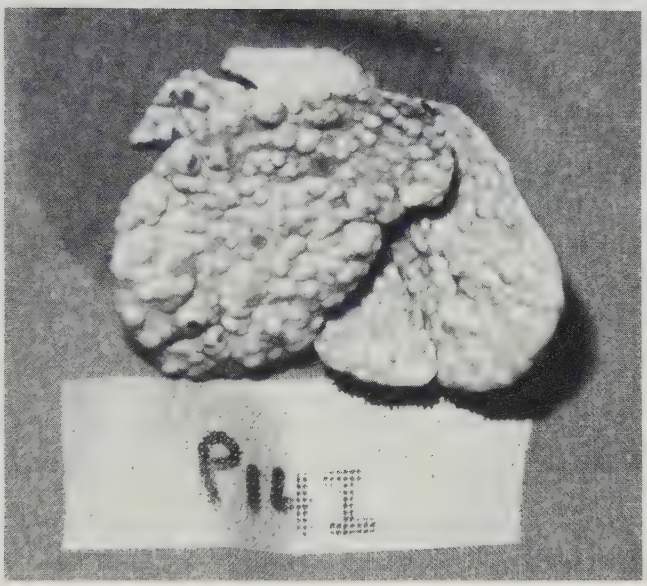


Fig.8. Macroscopic appearance of liver cirrhosis induced by thioacetamide for 12 weeks (0.5 g/l). Presented at Nordic Symposium. Metabolism of trace elements related to human diseases. Loen, Norway, June 1985.

There was a pronounced cellular and nuclear atypia,. Cirrhotic animals also showed elevated levels of ALP (13.3 $\pm$ 2.3 vs 8.5 $\pm$ 1.7 µKat/l) β-NAG (21.7 $\pm$ 6.8 vs 8.5 $\pm$ 1.5, U/l) and β-Gluc (0.88 $\pm$ 0.12 vs 0.50 $\pm$ 0.06, U/l) compared to controls. Transaminases were unchanged.

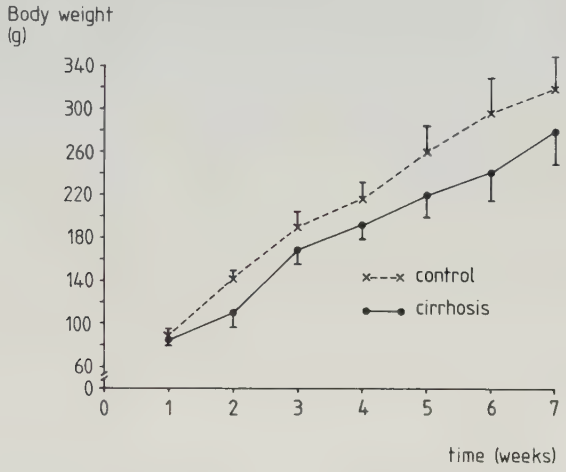
The idea of using different concentrations of thioacetamide was to see if a higher dose could accelerate the development of cirrhosis in older animals. We found that administration of 0.5 g/l of thioacetamide to large animals

(420-490 g) during 12 weeks led to the development of cirrhosis along with hepatoma and bile duct proliferation (unpublished observations, 1985), while administration of 0.3 g/l to smaller animals (150-160 g) induced cirrhosis to a similar extent but without formation of tumours. The formation of tumours may be caused by the higher dose or a higher susceptibility of tumour development in older rats.

Elevation of both lysosomal enzymes was noted in the present experiment in animals subjected to 0.5 g/l of thioacetamide. This could be due to decreased biliary excretion and/or increased production of lysosomal enzymes by activated macrophages. 0.3 g/l of thioacetamide led to decreased levels of β-Gluc in serum after 16 weeks of administration. Weber et al (1983) used the same dose for 22 weeks. They made similar observations. The reason for the different findings of increased serum lysosomal enzymes in the experiment with 0.5 g/l and unchanged or decreased levels with 0.3 g/l is not known.

The use of CCl_4 in experimental cirrhosis formation is well established, but the model of Proctor and Chatamra in our hands carries a high mortality rate (27%). We therefore wanted to assess another route of administration for a shorter period in young animals. We used young animals, since they are also sensitive to the hepatotoxic effect of

CCl_4 (McLean and MacLean, 1966; Slater, 1978; Trivedi and Mowat, 1983). With this model there was a mortality rate of 5%. The experimental animals lost some weight (fig.9.).



All surviving animals developed liver cirrhosis, which was of micronodular type (fig.10.)

The biochemical changes were as follows:

bilirubin (19.3±11.3 vs 3.1±0.6 mMol/l)

ALP (20.6±6.4 vs 8.5±1.7 µKat/l)

ASAT (13.5±5.0 vs 1.75±0.19 µKat/l)

ALAT (5.2±2.2 vs 1.1±0.14 µKat/l).

The repeated administration of CCl_4 and olive oil intraperitoneally induced liver cirrhosis in all surviving animals. There was no need for repeated anaesthesia, which probably reduced the mortality rate. The transaminases were elevated at sacrifice probably indicating on-going cell necrosis. The induction time was short and that can partly be explained by the use of young animals, since they have a high susceptibility for the development of liver injury.

It is not fully known if there is a spontaneuos reversibility of experimentally induced liver cirrhosis. The regenerative capacity of the cirrhotic liver is also not fully known. Some investigators have found that the cirrhotic liver regenerates as quickly as normal liver, while others have described a reduced regenerative capacity (Rabinovici and Wiener, 1961; Bengmark et al, 1966; Cameron and Karunaratne, 1963). It is also disputable whether the cirrhotic liver is replaced by normal tissue after partial hepatectomy (Rabinovici and Wiener, 1961; Cameron and Karunaratne, 1963). Liver tumours often arise in cirrhotic

livers and liver resections in such patients carry a high mortality and morbidity rate. A detailed knowledge about the conditions for regeneration in cirrhosis is therefore desirable.

We therefore wanted to study liver regeneration after repeated liver resections in CCl_4-induced cirrhosis. As in experiments above we had a 25% mortality with the model of Proctor and Chatamra. After the first liver resection there was a mortality of 33 percent and after the second liver resection 40 percent of the rats died. All rats showed at the time of first resection micronodular cirrhosis in resected specimen on microscopic examination (fig.11a, b).

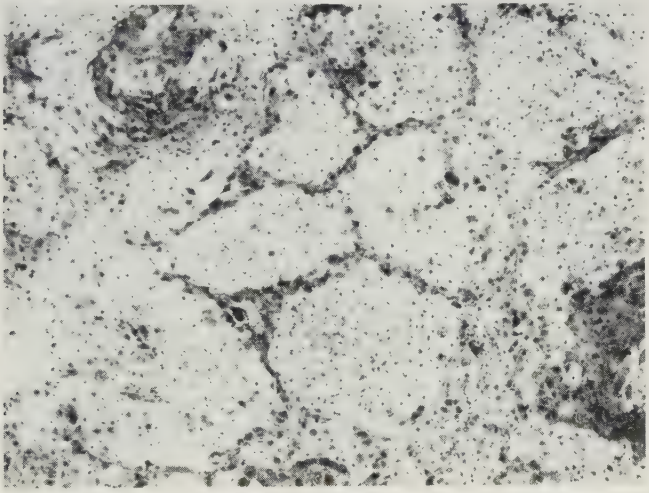


Fig.11a. Liver at the first resection. Cirrhosis with abnormal nodules and irregularly shined hepatocytes (htx-e /70) (V).

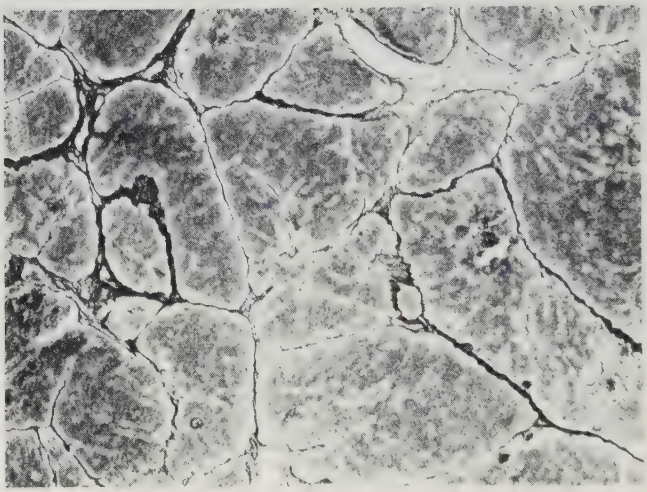


Fig.11b. Liver att the first resection. Cirrhosis with abnormal nodules separated by reticulin strands (reticulin/70) (V)

At the second resection (i.e., after one resection) all rat livers showed a quite different appearance with scattered reticulin strands combining portal tracts or portal tracts and central veins (fig. 12a, b).

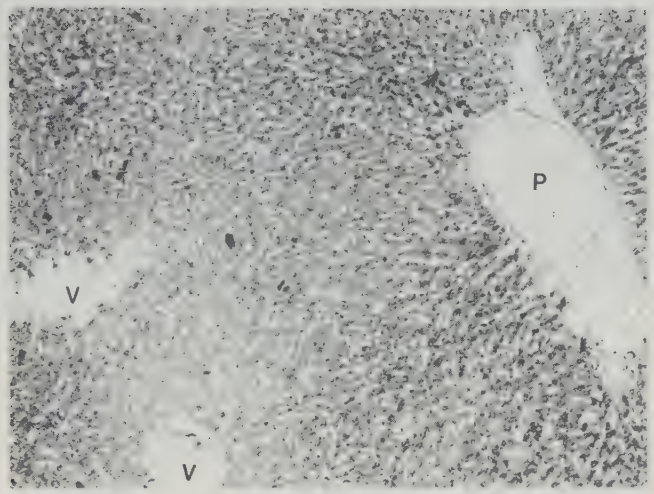


Fig.12a. Liver after the second resection. Normal appearing liver tissue, portal tract (P) to the right, central veins (V) to the left (htx-e /70).

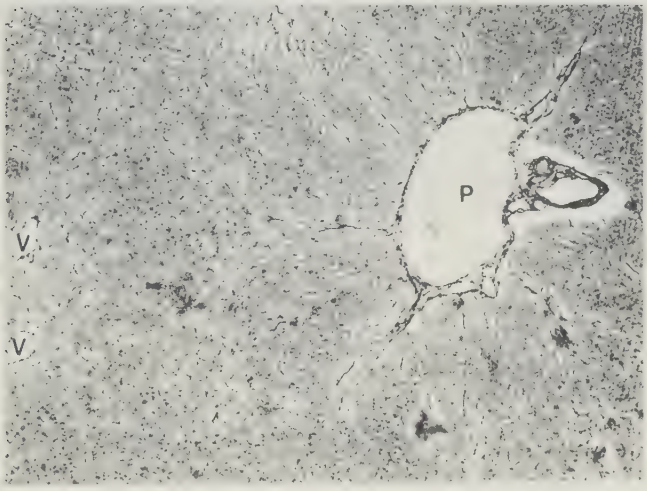


Fig.12b. Liver after the second resection. Normal appearing liver tissue, portal tract (P) to the right, central veins (V) to the left (reticulin/70).

These changes were uniformly distributed. At sacrifice (i.e. 6 weeks after second resection) all livers appeared normal in haematoxylin-erythrosine staining in all rats, while some abnormal reticulin strands were observed in silver stain (fig.13a, b).

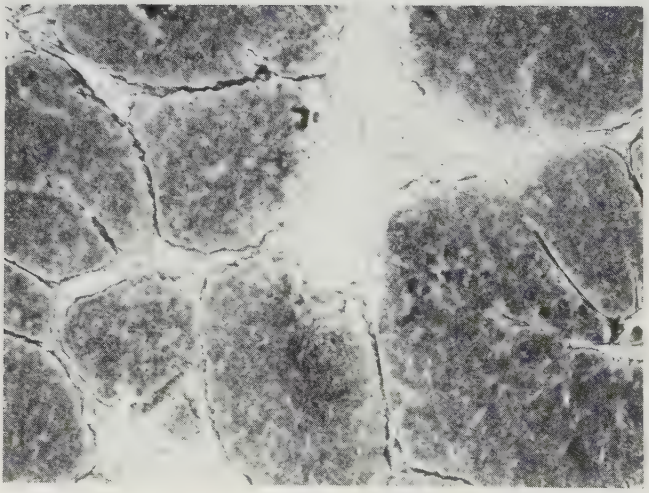


Fig.13a. Liver at the first resection. Cirrhosis with abnormal nodules separated by reticulin strands (reticulin/70).

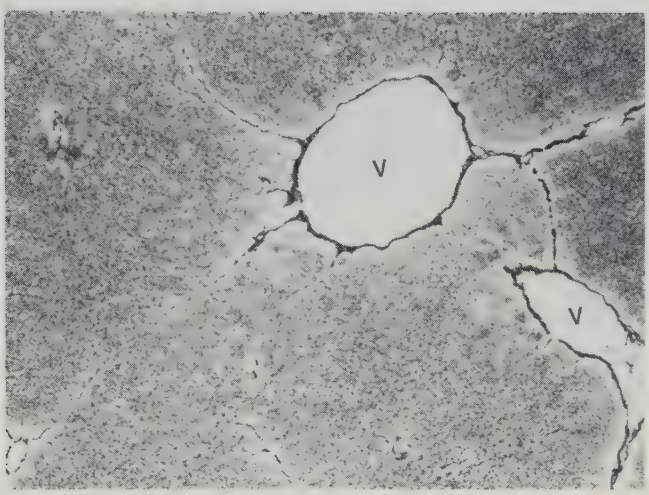


Fig.13b. Liver at sacrifice. No diffuse cirrhosis, but abnormal reticulin strands between central veins (V) (reticulin/70).

The control rats exposed to CCl_4 without resection showed at sacrifice micronodular cirrhosis similar to resected rats at first operation. Thus there was no indication of spontaneous regression. We found in this study after serial partial hepatectomies a regrowth of macro- and microscopically almost normal liver tissue with scattering of abnormal collagen on reticulin-staining compared to unresected control rats or resected liver tissue at the first and second resection.

Previous experiments on liver cirrhosis in rats have shown unexpectedly good regenerative capacity. The cirrhotic liver has been reported to regenerate completely after

partial hepatectomy (Rabinovici and Wiener, 1961; Islami et al, 1958). These studies have also found evidence that the cirrhotic liver is replaced by normal liver tissue after partial hepatectomy. In older studies a severely impaired regeneration after resection in cirrhosis has been found (Cameron and Karunaratne, 1936; Reifferscheid et al, 1959). However, the documentation of cirrhosis in these studies is not sufficient. In more recent studies a delayed regenerative response has been described with some remaining fibrosis in one study, but in another regeneration measured by uptake of tritiated thymidine was normal, but with some remaining fibrosis (Bengmark et al, 1966; Pechet and MacDonald, 1961). In these studies an experimental model of nutritional cirrhosis was used and it is possible that the cirrhosis induced in this way has other characteristics than toxin-induced cirrhosis. There are reports that toxin-induced cirrhosis to some extent are reversible (Quinn and Higginson, 1965). The reversibility seems to be more frequent when short induction times are used. In this experiment the unoperated cirrhotic controls were all cirrhotic at sacrifice i.e., 12 weeks after cessation of CCl_4-administration.

2. TRACE METAL CHANGES IN LIVER INJURY AND CIRRHOSIS (I, II, III, IV, V, VI, VII)

Administration of one dose of thioacetamide resulted in profound liver injury and alterations in the plasma and liver contents (I) of trace elements. The changes can be seen in table 2. Plasma zinc decreased at 24 h, but returned to normal at 48 h. Plasma copper did not change at these time points. The concentration of zinc in the livers of the thioacetamide group showed a significant increase 24 h after administration, and this returned to normal after 48 h. The copper content in the livers of the thioacetamide group increased significantly after 24 h.

In zinc treated animals, the plasma level of zinc was elevated after 48 h compared to controls, while copper plasma level decreased significantly and the zinc and copper content in the liver increased.

Patients with liver cirrhosis have been reported to be zinc depleted, and the low zinc levels in plasma and liver tissue have been thought to be of pathogenetic importance for the development of cell injury in human viral hepatitis, alcoholic liver cirrhosis and in cirrhosis of rats after CCl_4 administration (Henkin and Smith, 1972; Sinha and Gabrieli, 1970; Kahn and Ozeran, 1967). In human liver cirrhosis plasma zinc levels are decreased and plasma

Table 2. Trace elements in the plasma and liver of control and experimental animals 24 h and 48 h after administration of thioacetamide with or without zinc. (mean ± S.D.)

	Control n=6	Thioacetamide 24 h n=6	Thioacetamide 48 h n=6	Thioacetamide 48 h + zinc n=10
Plasma				
Zinc(μg/ml)	1.76±0.20	1.5±0.13**	1.90±0.60	2.0±0.3**
Copper(μg/ml)	1.20±0.10	1.06±0.28	1.2±0.20	0.8±0.3**
Liver				
Zinc(μg/g)	28.7±1.18	38.45±4.1**	27.95±2.2	37.9±9.7
Copper(μg/g)	5.27±0.45	7.36±0.4**	6.45±1.35*	8.5±1.4**

*denotes $p<0.05$ compared to control rats.

**denotes $p<0.01$ compared to control rats.

copper levels are increased. Copper:zinc ratio in the plasma was increased by 66% in cirrhotic patients when compared to control in one study (Dashti et al, 1984). These changes are thought to be mediated via the leukocyte endogenous mediating factor (LEM), which is released from phagocytes during inflammatory processes (Pekarek et al, 1972). In this acute experiment the decrease in plasma zinc at 24 h may reflect an increased liver uptake. The increased liver zinc content may be of importance for the repair process following cell injury, since zinc is essential for DNA synthesis and liver protein metabolism. Plasma and liver zinc levels increased by zinc administration, and in these animals there were indications of reduced cell injury regarding reduced release of lysosomal enzymes. Zinc thus seemed to have some effect on thioacetamide-induced hepatic toxicity. Besides the effect of zinc mentioned above, the scavanging effect of zinc as part of SOD may also be of pathophysiologic importance. Plasma levels of copper were unchanged after thioacetamide administration. We found however, an increase in the level of copper in liver after thioacetamide administration both at 24 and 48 h. The cause of this increase is unknown. Cain and Griffiths (1984) have found that during liver regeneration liver zinc content was high within 14 h while liver copper content was elevated during the whole regenerative process. An increased synthesis of cerulo-

plasmin in the liver might be the cause of the high content of copper in the liver (Ritland et al. 1979).

We studied changes of zinc and copper in experimental cirrhotic models (II, III, IV, V, VI).

In thioacetamide-induced liver cirrhosis we could observe a decrease in plasma zinc and copper (table 3).

Table 3

Plasma zinc and copper in experimental and human alcoholic cirrhosis

	Zinc (µg/ml)		Copper (µg/ml)	
	Control	Cirrhotic	Control	Cirrhotic
CCl_4				
(IV)	1.4 ±0.1	0.9±0.1**	1.5±0.3	2.0±0.5*
(V)	1.8 ±0.1	1.5±0.15*	1.1±0.34	1.5±0.7*
Thioacet.				
(III)	1.5±0.2	1.15±0.1*	1.4 ±0.2	1.0±0.1*
(II)	1.4±0.1	1.13±0.1*	1.5 ±0.3	1.04±0.1*
Human	Reference value (µmol/l)		Reference value (µmol/l)	
(VII)	9.9 - 17.1	11.95	14.2 -23.6	18.7

* denotes $P < 0.05$ compared to control animals

** denotes $P < 0.01$ compared to control animals

*** denotes $P < 0.005$ compared to control animals

In one study (II) both plasma levels of magnesium and selenium decreased significantly. The liver zinc and copper content increased significantly in thioacetamide-induced cirrhosis (table 4).

Table 4

Liver and copper contents in experimental cirrhosis

	Zinc (µg/g)		Copper (µg/g)	
	Control	Cirrhotic	Control	Cirrhotic
CCl_4				
(III)	20.5 ±4.6	12.7 ±3.4**	3.2 ±0.6	5.7 ±1.7**
(IV)	20.6 ±4.6	14.3 ±2.6**	3.4 ±0.7	5.8 ±1.9**
(V)	24.2 ±3.5	19.1 ± 2.4*	4.5 ±1.4	5.4 ±1.8
Thioacet.				
(III)	21.6 ±5.0	52.0 ±8.0***	3.4 ±0.7	7.2 ±1.5***

* denotes $P < 0.05$ compared to control animals

** denotes $P > 0.01$ compared to control animals

*** denotes $P < 0.005$ compared to control animals

In carbon tetrachloride-induced liver cirrhosis, the alterations of trace elements were somewhat different. As it is seen from table 3 the plasma zinc decreased and plasma copper increased significantly. Liver zinc content decreased significantly and copper liver content increased significantly in carbon tetrachloride-induced liver cirrhosis (table 4).

Low plasma zinc and high plasma copper have been observed in different liver diseases such as human alcoholic liver cirrhosis, viral hepatitis and CCl_4-induced cirrhosis in rats (Vallee et al, 1956; Sinha and Gabrieli, 1970; Dashti et al, 1986; Henkin and Smith, 1972; Kahn and Ozeran, 1967). We also found decreased plasma zinc levels in thioacetamide-induced cirrhotic rats, but copper levels were also low.

Liver zinc content decreased in rats with CCl_4-induced liver cirrhosis as has been reported by others (Vallee et al, 1957; Kilerich et al, 1980). The liver zinc content of thioacetamide rats increased continuously over time however. The reason for this difference is not known, but may reflect different mechanisms of action of the two hepatotoxins and it is important to recognize this when evaluating results obtained with the two models of cirrhosis. The hepatic copper content was significantly

increased in both groups of cirrhosis. Increased liver copper content has also been found in patients with primary biliary cirrhosis, long standing biliary obstruction, viral hepatitis and chronic active liver disease (Ritland et al, 1977; Henkin and Smith, 1972). The significance of this increase for the development of cirrhosis is not obvious.

In order to further study the behaviour of liver zinc, we looked at plasma and liver zinc after repeated liver resections (V). As in the other experiments plasma zinc levels decreased during the development of cirrhosis and the decrease was further pronounced after resection (table 5).

Table 5

Plasma and liver zinc in cirrhotic and control rats.

	Control (6)	Cirrhosis (6)	After 1st resection (6)	After 2nd resection (6)
Plasma (μg/ml)	1.81±0.10	1.50±0.15*	1.57±0.20*	1.05±0.11**
Liver (μg/g)	24.2±3.5	19.1±2.4*	33.5±2.6*	41.6±7.4*

Values are given as $\bar{x}$ ± (S.D.) for number of rats within parenthesis

* $p < 0.05$ compared to control rats.

** $p < 0.01$ compared to control rats.

Simultaneously liver zinc content increased significantly. The liver zinc content in cirrhotic animals was low before start of resection. The plasma copper was high in cirrhotic rats and increased after resection. The liver copper content was unchanged in all animals.

It is not known if the low zinc content is primary or secondary to the cirrhosis and if hepatic depletion bears any relation to the development of cirrhosis. The successive increase in hepatic zinc content and decrease in plasma zinc after repeated resections may indicate an increased demand of the regenerating liver but also a normalization of the hepatic parenchyma (Fujioka and Lieberman, 1964; Vallee, 1976). A recent experimental study seems to show that zinc has a direct effect on CCl_4-induced collagen formation by inhibiting proline, which mediates a crucial step in the collagen synthesis (Anttinen et al, 1984). Plasma copper levels increased in cirrhotic rats and a further increase was noted after resection. The increase could be partly explained by the decrease in plasma zinc, since they share common binding to albumin (Giroux and Schoun, 1981). It could also be secondary to generation of free radicals since copper plays a role in the regulation of free radical metabolism (Giamaolo et al, 1982). Copper availability is essential for the synthesis of mature collegen and elastin (Harris et al, 1980). Impaired collagen synthesis has been described in copper deficient

animals (Giamaolo et al, 1982).

It thus was evident that the increase in liver zinc content parallelled the normalization of histological changes. We therefore wanted to see if zinc supplementation to rats with CCl_4 administration could normalize liver zinc content and thereby reduce or prevent the formation of cirrhosis (VI). When zinc sulphate was administered concomittantly with CCl_4, liver zinc content was normal, while the copper content was elevated compared to control animals (table 6)

Table 6

Zinc and copper contents in liver. Values are given as $\bar{x} \pm SD$.

	Control n=6	Group 1 n=4	Group 2 n=6
Zinc (µg/g)	20.6 ± 4.7	12.9 ± 7.2	18.4 ± 9.2
Copper (µg/g)	3.3 ± 0.7	8.1** ± 4.0	8.8* ± 4.6

* denotes $P < 0.05$ compared to control animals

** denotes $P < 0.01$ compared to control animals

There was a mortality rate of 50%, and 4 out of 6 surviving animals showed cirrhosis, while in control animals i.e. animals subjected to alcohol and CCl_4 without zinc administration, the mortality rate was 64% and 4 surviving rats were cirrhotic.

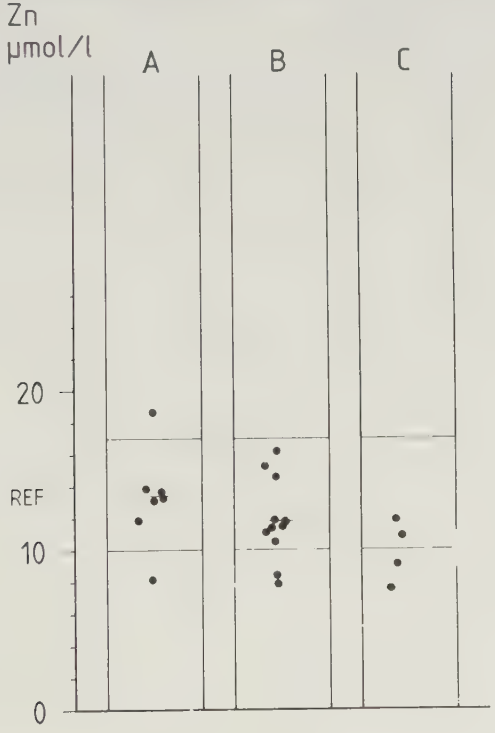
It has been described that zinc ions have a protective effect against liver injury. Ludwig and Chvapil (1982) found that pretreatment of rats with 2 mg of zinc chloride per 100 g of body weight intraperitoneally for 3 consecutive days resulted in 50 percent reduction of CCl_4-induced centrilobular necrosis along with significant reduction of β-Gluc in zinc treated animals. Anttinen et al (1984) suggested that peroral zinc treatment has a direct and selective inhibitory effect on carbon tetrachloride-induced collagen accumulation in rat liver.

We almost normalized the liver zinc content in cirrhotic rats by zinc administration. This seemed to reduce the mortality rate and the induction of cirrhosis. The number of animals is however, too small to draw any definite conclusions.

Hypozincaemia has also been suggested to play a role in the development of hepatic encephalopathy in cirrhotic patients (Reding et al, 1984). It was shown in this study that short-term oral supplementation of zinc (600 mg/day for 7

days) to patients improved chronic encephalopathy. One mechanism of action may be that zinc is important for the function of ornithine transcarbamylase, an enzyme important for the urea synthesis and detoxification of ammonia.

In our material of 22 patients with alcohol-induced liver cirrhosis and a histoory of hepatic encephalopathy, we found that plasma zinc was somewhat lower in patients belonging to Child's group C compared to group A, although not reaching statistical significance (fig.14).



Ammonia levels were elevated in 16 out of 19 patients evaluated. The patients were also carefully evaluated by psychometric tests (table 7).

In 4 out of 15 tests used the cirrhotic patients exhibited a poorer performance than the reference group of 50 healthy persons.

Upon multiple correlation testing there was no statistically significant correlation in the cirrhotic patients between zinc concentrations in plasma and any of the psychometric tests.

In liver cirrhosis low zinc levels in plasma and liver tissue have been observed (Vallee et al, 1956; 1957). This may be due to several factors such as malabsorption, malnutrition and hyperzincuria. Alcohol by itself has also an effect on the renal tubules which further increases the loss of zinc (Prasad, 1983). The low albumin levels in cirrhotic patients will further contribute to the low zinc levels.

The role of zinc in the pathogenesis of liver cirrhosis is not clear. Zinc administration seems to protect the liver cells from the toxic action of carbon tetrachloride (Ludwig and Chvapil, 1982) and thioacetamide as mentioned earlier. Since zinc is also important for normal collagen synthesis, it is possible that the hypozincaemia may contribute to the fibrosis. Zinc also participates in many enzyme systems

Table 7

Psychometric test scores in the 50 normal control subjects and the 22 cirrhotic patients (median interquartile range).

Psychometric test	Control Subjects n = 50	Cirrhotic Patients n = 22	P
SRB:1	23 (20-25)	21 (18-26)	n.s.
SRB:2	20 (18-24)	18 (14-23)	$p < 0.05$
SRB:3	25.5 (21-30)	21.5 (10-25)	$p < 0.01$
MFD	2 (1-4)	4 (2-8)	$p < 0.05$
BENR	7 (6-8)	6 (4-6)	$p < 0.001$
BENW	4 (2-7)	7 (5-9)	$p < 0.001$
WP	25 (20-27)	21 (18-25)	n.s.
DIG	39 (31-44)	34.5 (27-44.5)	n.s.
DOTT	415.5 (373-459)	482 (410-536)	$p < 0.05$
DOTW	14 (8-20)	23 (20-26)	n.s.
DOTF	22 (16-27)	12 (6-18)	n.s.
CWT	130 (116-153)	131 (115-161)	n.s.
CYL	74 (54-78)	69 (61-78)	n.s.
TMT	116 (103-132)	137 (106-108)	n.s.
TMD	23 (15-31)	19 (11-51)	n.s.

involved in carbohydrate and protein metabolism, and it is reasonable to assume that the low zinc levels in plasma and liver may play a role in the metabolic disturbances in liver cirrhosis. Recently the low zinc levels have been shown to be related to the development of encephalopathy (Reding et al, 1984), and administration of zinc to patients with encephalopathy improved the performance of trailmaking test concomittant with an increase of BUN. However, no information was given regarding ammonia levels. We found in this group of patients with stable liver cirrhosis and a history of encephalopathy that plasma zinc was somewhat decreased. A majority of the patients also had hyperammonemia. However, there was no correlation between zinc and NH levels. However, most of our patients had a good hepatic metabolic capacity and this may be one explanation for the lack of correlation. It may only be seen in decompensated phases of the liver disease.

The hepatic encephalopathy syndrome is complex and several pathogenetic mechanisms are probably involved. If there is any role of hypozincaemia it is only as a minor contributing factor.

3. The role of free radicals in liver cell injury (VIII, IX).

The addition of ethanol to the mononuclear leukocyte cell suspensions led to a significant increase in ADPRT activity at the concentrations of 50 and 100 µl (1mM and 2mM) but not 150 µl (3mM) (fig.15) (VIII).

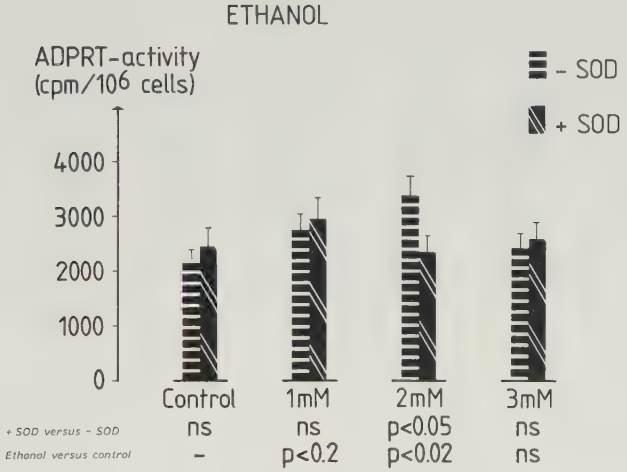


Fig.15. Mononuclear leukocytes (0.5×10^6) exposed to 1 (50 µl), 2 (100 µl), and 3 (150 µl) mM concentrations of ethanol. Values are given as mean SEM. Dashed fields denote cell suspensions without SOD and black fields with 200 µg SOD added.

When carbon tetrachloride was added, there was a significant increase in enzyme activity at the concentrations of 0.1 mM and 0.25 mM but not at higher concentrations (fig.16).

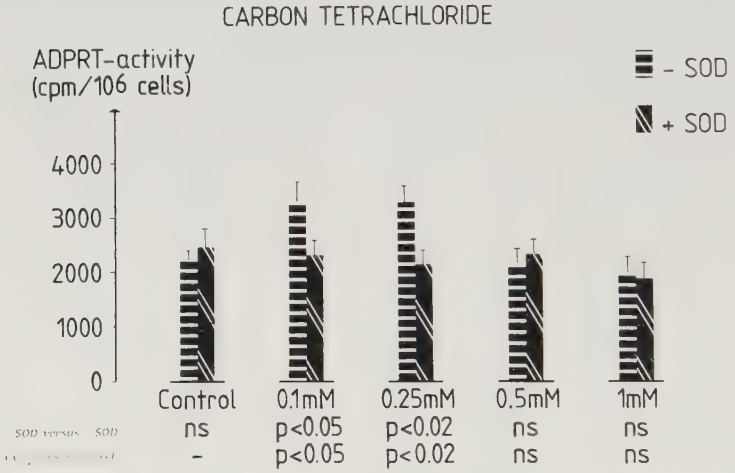


Fig.16. Mononuclear leukocytes (0.5 x 10^6) exposed to 0.1, 0.25, 0.5 and 1mM carbon tetrachloride. Further details as in fig. 15.

Thioacetamide finally caused an increased activity at 1mM and 10mM concentrations, but not at a concentration of 0.1mM (Fig.17).

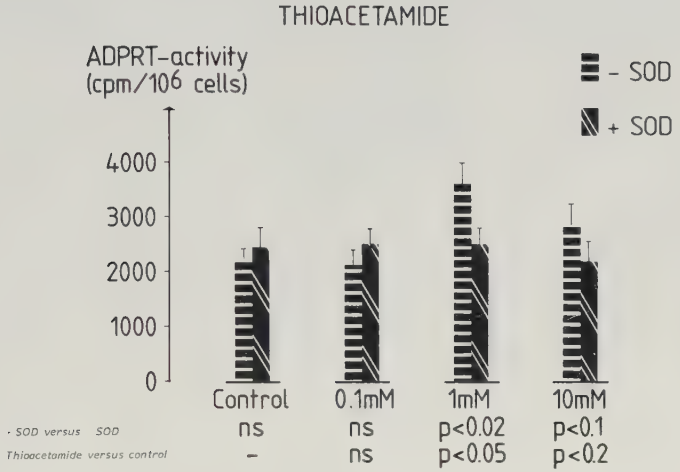


Fig.17. Mononuclear leukocytes (0.5 x 10^6) exposed to 0.1, 1, and 10 mM thioacetamide. Further details as in fig.15.

The addition of the highest concentration of ethanol and carbon tetrachloride caused widespread cell death, verified at histologic examination. SOD protected the cells from the damage at the concentration where ADPRT induction occurred (Fig. 15-17).

It is well known that the metabolism of CCl_4 involves the production of free radicals (Slater and Sawyer, 1971), and repeated CCl_4 intoxication induces liver cirrhosis. Thioacetamide, which is another well-known hepatotoxin produces liver cirrhosis and increases DNA synthesis in hepatocytes (Morley et al, 1977; Rather, 1951). It is known in both humans and animals that lipid peroxidation increases after alcohol ingestion (Plaa and Witschi, 1976; Torrielli et al, 1978). Other investigators have suggested that lipid peroxidation is implicated in the pathogenesis of alcoholic liver disease such as cirrhosis (Videla and Valenzuela, 1982).

Oxygen radicals are very potent in inducing DNA strand breaks (Ward, 1977) (Fig.18).

Adenosine diphosphate ribosyl transferase (ADPRT) is a chromosome associated enzyme which transfers ADP-ribose moities from NAD to nuclear protein important for regulating DNA damage and repair (Hilz and Stone, 1976;

Hayaishi and Ueda, 1977; Purnell et al, 1980; Berger et al, 1980; Miwas et al, 1983).

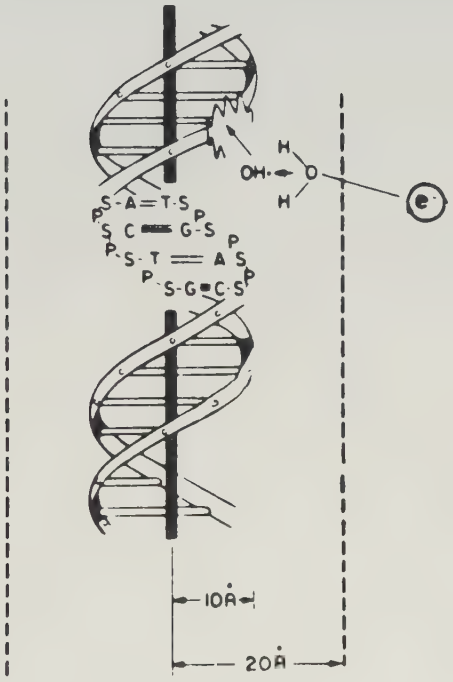


Fig.18. Free radical induced DNA strand breaks.

The enzyme is also supposed to be involved in cellular differentiation and gene expression (Farzanah et al, 1982; Johnstone and Williams, 1982; Pero et al, 1985; Althaus et al, 1982). It is also dependent on DNA and histones and strongly stimulated by DNA strand breaks (Tsopanakis et al, 1978; Juarez-Salinas et al, 1979; Benjamin and Gill, 1980; Durkacz et al, 1980; Cohen and Berger, 1981; Pero et al, 1983; Jacobson et al, 1983; Jonsson et al, 1985; Berger, 1978). ADPRT activity was used as an indicator of oxygen radical induced DNA damage following exposure of cells to alcohol, CCl_4 and thioacetamide.

The data recorded (fig. 15-17) clearly show that oxygen radicals are produced during a standardized exposure to these agents, and that the cell injury could be prevented by the addition of scavangers. Consequently, these data may implicate a role for oxygen radicals in cirrhotic disease. Some precautions must be made, however, on how relevant results obtained from suspended leukocytes are to hepatocytes. The leukocytes have low levels of endogenous superoxide dismutase compared to liver cells and may thus be more susceptible to superoxide mediated damage. On the other hand they lack some of the enzyme systems involved in the activation of certain hepatoxins. The effect of SOD is striking in the system tested and a preventive action of oxygen scavenging on hepatic cell injury and formation of

cirrhosis may be suggested.

It was therefore of interest to study changes of ADPRT and the role of superoxide dismutase in the prevention of the toxicity of carbon tetrachlorid-induced liver injury in animals (IX).

Plasma bilirubin, ALP, ASAT, β-Gluc and β-NAG increased significantly in rats exposed to CCl_4 (table 8).

The increase of bilirubin, ASAT, ALAT and β-Gluc was significantly reduced when SOD was administered together with CCl_4. ALP and β-NAG was also somewhat reduced after SOD. There was a small elevation of ADPRT in animals receiving CCl_4, although it was not reaching statistical significane with sample sizes of n=6. Histologically centrilobular necrosis and hepatocytic swelling were observed in all animals receiving CCl_4, but the necrotic lesions were less widely spread and the nuclear lysis was somewhat delayed in animals given SOD (Table 9, Fig.19a, b).

Table 8

Liver function tests, lysosomal enzymes and ADPRT levels for CCl_4 administration with or without SOD. Values are given x $\pm$ S.D for number of rats within parentheses.

	Control (6)	Control+SOD (6)	CCl_4 (6)	CCl_4+SOD (6)
Bilirubin (µmol/l)	6.6 $\pm$ 2.0	8.1 $\pm$ 3.4	12.8 $\pm$ 2.1**	8.0 $\pm$ 2.9****
ALP (µkat/l)	9.7 $\pm$ 1.3	11.7 $\pm$ 3.3	18.3 $\pm$ 8*	16.0 $\pm$ 2.8
ASAT (µkat/l)	1.5 $\pm$ 0.24	1.8 $\pm$ 0.36	35.5 $\pm$ 3.5**	22.6 $\pm$ 8.8***
ALAT (µkat/l)	0.98 $\pm$ 0.33	0.57 $\pm$ 0.28	12.7 $\pm$ 0.44**	8.0 $\pm$ 4.0***
β-Glucuronidase (u/l)	0.36 $\pm$ 0.05	0.57 $\pm$ 0.28	1.72 $\pm$ 0.44**	0.79 $\pm$ 0.37****
β-hexosaminidase (u/l)	13.5 $\pm$ 2.6	14.8 $\pm$ 2.4	34.4 $\pm$ 18.0*	23.1 $\pm$ 13.1
ADPRT cpm/1 ml blood	140 $\pm$ 83.4	125 $\pm$ 44.3	172 $\pm$ 87.3	155 $\pm$ 53.6

* $P < 0.05$ compared to control rats

** $P < 0.01$ compared to control rats

*** $P < 0.05$ compared to CCl_4

**** $P < 0.01$ compared to CCl_4

Table 9

Necrotic areas on histologic examination

+	= 1/3 of total area necrotic
++	= 1/2 of total area necrotic
+++	= 2/3 of total area necrotic

CCl_4	CCl_4 + SOD
++	+++
+++	+
+++	+
+	+
++	+
+++	++

Administration of SOD together with CCl_4 seems to reduce the cell damage. The mechanism of CCl_4-induced liver cell necrosis is considered to be due to enzymatic activation of CCl_4 to CCl_3 free radical followed by peroxidation and progressive destruction of membrane phospholipids of the endoplasmic reticulum in the liver (Reynolds and Ree, 1971). The generation of free radicals induces DNA damage, which is reflected in an increased ADPRT activity. The hepatic cell necrosis in this study was also evidenced by an increased release of ALAT and lysosomal enzymes. A possible relationship between lipid peroxidation and a

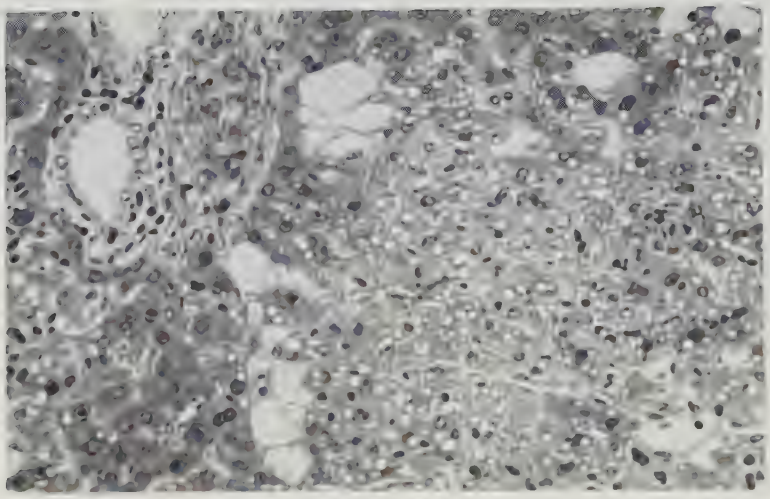


Fig.19a. Liver from rat after CCl_4-administration. Portal tract upper left, hepatic vein lower right. 2/3 of the parenchyma around the vein is necrotic, some foamy, swollen hepatocytes in the periphery of the necrosis (htx-e /280).

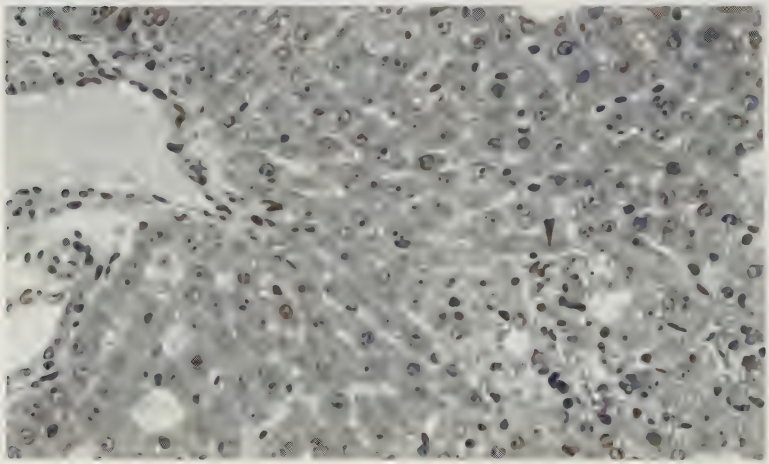


Fig.19b. Liver from a rat after SOD and CCl_4-administration. Portal tract to the left. Groups of necrotic liver cells (arrow) to the right and dispersed swollen, foamy hepatocytes (htx-e /280).

release of lysosomal enzymes as markers of CCl_4 injury after CCl_4 exposure has previously been described in an in-vitro study (Younes et al, 1983).

Superoxide dismutase is a scavanger of superoxide anion radicals which are generated among others by CCl_4. SOD levels increase following CCl_4 administration (Adachi et al, 1983). Oxygen radicals have been implicated in inflammatory injury and ischaemia, and in these conditions pretreatment with SOD and other scavangers have been shown to reduce the extent of injury (McCord, 1974; Schönberg et al, 1983). The administration of SOD together with CCl_4 seems to reduce the acute hepatic cell injury. This implicates a role of free radicals in the acute hepatotoxic injury of CCl_4. If similar pathogenetic mechanisms are involved in the development of liver cirrhosis after chronic CCl_4 exposure, remain to be shown.

Thus, all flesh progresses toward the sackcloth of salt, the ashfruit of our wakes, the dwarf rose of your desert sand, and the bride of night escorted to the door, before the dawn...

From Exile,
by Saint-John Perse (1887-1975)
Nobel Prize, 1960

F. SUMMARY

1. In the present studies we have reassessed experimental models of cirrhosis by the administration of thioacetamide and carbon tetrachloride.

Acute thioacetamide administration induced profound liver injury with elevations of transaminases and lysosomal enzymes. These alterations provoked us to investigate in more detail the effects of prolonged administration of thioacetamide mixed in drinking water and compare it to the more commonly used model of carbon tetrachloride administration, in order to find an optimal experimental model.

Thioacetamide in a dose of 0.3 g/l in drinking water produced liver cirrhosis of micronodular type in 75% of the animals after 16 weeks without mortality. By increasing the dose to 0.5 g/l and by using older animals, all animals developed liver cirrhosis at the end of 12 weeks, but this was associated with the formation of tumours and bile duct proliferations in some animals (unpublished observations). Administration of CCl_4 by different routes gave us different results. In our hands, intragastric admini-

stration of CCl_4 with weight-adjusted doses in animals pretreated with phenobarbitone had a mortality rate of 27% and an incidence of cirrhosis of 76% in surviving animals. Combination of alcohol and CCl_4 induced liver cirrhosis in all surviving animals but was associated with a mortality rate of 64%. Both these hepatotoxins induced micronodular cirrhosis (table 10).

CCl_4 (0.15 ml) mixed with olive oil was given intraperitoneally 3 times per week for 7 weeks, to see if it was possible to reduce the mortality rate and shorten the induction time. This led to a 5% mortality rate and 100% cirrhosis in all surviving animals.
We considered this model of CCl_4-administration mixed with olive oil to smaller animals to be rapid, reliable and reproducible. The cirrhosis bears several biochemical and histological similarities to human cirrhosis.

2. Human liver cirrhosis is associated with abnormalities of zinc and copper metabolism. After administration of thioacetamide plasma zinc decreased after 24 h and normalized after 48 h, liver zinc content increased after 24 h and normalized after 48 h. No changes were observed of plasma copper levels, while copper liver content increased after 24 and 48 h. Administration of zinc simultaneously with thioacetamide normalized the plasma and zinc content,

Table 10. Cirrhosis formation and mortality rates in experimental models.

Hepatotoxin	Weight of animals g	No. of rats	Admin.	Duration weeks	Mort. rate	Cirrhosis %-age
CCl_4 + Phenobarb. (III)	170-200	11	i.g.	16	3/11 26%	76
CCl_4 + o.o. (IV)	60-80	18	i.p.	7	1/18 5%	100
CCl_4 + o.o + alc. (VI)	150-170	11	i.g.	8	7/11 64%	100
CCl_4 + o.o. + alc. + zinc (VI)	150-160	12	i.g.	8	6/12 50%	66
Thioacet. (0.5g/l)(II)	420-490	9	p.o.	12	0/9 0%	100
Thioacet. (0.3g/l)(III)	150-160	8	p.o.	16	0/8 0%	75

i.g. = intragastrically

i.p. = intraperitoneally

p.o. = perorally

o.o. = olive oil

and decreased the plasma copper level, but did not effect the cell damage. Trace metal changes were also studied in the models of liver cirrhosis with thioacetamide and carbon tetrachloride. In carbon tetrachloride-induced liver cirrhosis all animals showed decreases in plasma zinc and increases in plasma copper which is in agreement with findings in humans and other models of experimental cirrhosis (table 3). Liver zinc content decreased and liver copper content increased (table 4). In thioacetamide-induced cirrhosis both zinc and copper were decreased in plasma, while the liver zinc content was increased (table 3, 4). The different alterations of these trace elements may indicate a different mechanism of action of these two hepatotoxins.

We also found a successive increase in hepatic zinc content and decrease in plasma zinc after repeated resections in cirrhotic animals which indicate an increased demand of the regenerating liver for zinc. Plasma copper levels increased in cirrhotic rats and a further increase was noted after resections. The liver copper was unchanged in all animals.

Administration of zinc sulphate concomittantly with carbon tetrachloride normalized the liver zinc content and increased the liver copper content (table 6). This was followed by a reduced incidence of cirrhosis and mortality rate, but the histologic picture was not altered compared

to cirrhotic rats without zinc administration.

The importance of hypozincaemia for the development of encephalopahty in cirrhotic patients remains obscure. We could not find any correlations between plasma zinc levels and 12 psychometric tests used in 22 patients with alcohol induced cirrhosis and a history of encephalopathy. Plasma zinc levels were decreased in patients of Child's C.

3. The injurious action of alcohol, CCl_4 and thioacetamide was investigated in two studies. The addition of these hepatotoxins to cell suspensions of human mononuclear leukocytes led to a significant increase in ADPRT (used as an indicator of oxygen radical induced DNA damage) (fig.20).

Administration of SOD completely prevented this toxic effect. This study led us to administer SOD along with CCl_4 to rats. We found then a significant reduction in the injurious action of CCl_4 evidenced by reduced enzyme release and extent of cell necrosis. These data may implicate a role of oxygen free radicals in CCl_4-induced liver cell injury.

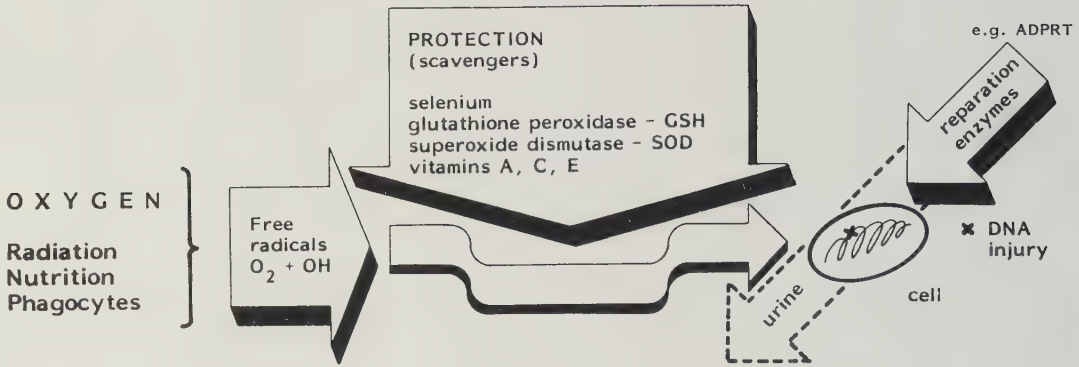


Fig.20. The role of free radicals and scavengers in cell injury.

Here and there, a sheltering tree
beside a lonely house in a desert
(dry and full of ravens; in the midst of the lush greenery
its trunk stands hollow, gray, withered,
with a dead raven hanging
by one pinion from a broken bough
and other ravens, still alive, sitting solemnly
before it, not daring to peck at the corpse).

From Space
by Juan Ramón Jiménez (1881-1958)
Nobel Prize, 1956

G. CLINICAL IMPLICATIONS AND FUTURE ASPECTS

The optimal model of experimental liver cirrhosis is still lacking. We have reassessed the use of thioacetamide in a new mode of administration and found it superior to CCl_4 in some aspects. The differences in trace element alterations need further evaluation, especially for studies of prevention.

The increased rate of tumour formation with increasing dose of thioacetamide is of interest with regard to trace metal changes and development of histological alterations. Few long term studies have been performed to investigate the reversibility of the experimental liver cirrhosis. CCl_4-induced liver cirrhosis seems to disappear with discontinuation of CCl_4, but this is not known in thioacetamide-induced cirrhosis.

A careful study of collagen metabolism in rats given both hepatotoxins and zinc must be performed, to study any

preventive effect on cirrhosis formation. With thioacetamide any effect of zinc administration on tumour formation is also of interest.

It appears that rat livers with CCl_4 induced cirrhosis exhibit a good regenerative capacity after repeated resections with normalization of histological changes. Similar studies should be performed in thioacetamide-induced cirrhosis. It is worthwhile trying to estimate the serum procollagen peptides in cirrhotic and resected animals to help to distinguish between fibrotic and non-fibrotic liver disease, and to see if zinc supplementation to resected animals can correct the alterations of trace elements and enhances the cirrhotic liver to regenerate by estimating the serum procollagen peptides.

Other regenerative stimuli must also be used, such as portal vein branch ligation in cirrhotic animals to see if a similar reaction can be elicited, and with estimation of trace elements, lysosomal enzymes and histological patterns. This may have clinical implication in cirrhotic patients.

The role of zinc for the development of encephalopathy should be studied by zinc administration to cirrhotic patients under careful evaluation by well designed psychometric tests. Concomittant alterations of ammonia

detoxification and collagen metabolism should be estimated.

We have demonstrated an involvement of oxygen free radicals in the pathogenesis of liver cell injury following hepatotoxins, and we observed a preventive effect of SOD in an acute experiment. It is worthwhile to see if chronic administration of SOD can prevent the injurious effect of CCl_4 in the development of liver cirrhosis.

There is a force that withers the soul
a handshake of brick, a breath of dust.
There is a force that withers the song.
There are desert dwellers who are but sand.

From The Grass in Thule
by Harry Martinsson (1904-1979)
Nobel Prize, 1974

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Open your house for me,
open all doors, all gates for me,
blowing in like a gale.

When I sweep into your house
there shall no longer be room for you,
you shall live in the desert as an outcast.
I shall drive you out into the desert,
you shall lie naked in the desert under the stars.

But I shall live in the house that was yours,
I shall pervade it.

From Evening Land,
by Pär Lagerkvist (1891-1974)
Nobel Prize, 1951

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